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=> s acyl carrier protein and non-radioactive label?
L1            0 ACYL CARRIER PROTEIN AND NON-RADIOACTIVE LABEL?

=> s acyl carrier protein and chromo? label?
L2            0 ACYL CARRIER PROTEIN AND CHROMO? LABEL?

=> s acyl carrier protein and chromophore
L3           17 ACYL CARRIER PROTEIN AND CHROMOPHORE

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4            5 DUP REM L3 (12 DUPLICATES REMOVED)

=> d l4 1-5 ibib ab

L4   ANSWER 1 OF 5     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2001301510     MEDLINE
DOCUMENT NUMBER:    21114173   PubMed ID: 11162232
TITLE:              The product of dpsC confers starter unit fidelity upon the
                    daunorubicin polyketide synthase of Streptomyces sp. strain
                    C5.
AUTHOR:             Rajgarhia V B; Priestley N D; Strohl W R
CORPORATE SOURCE:   Department of Microbiology, Ohio State University, 484 West
                    12th Avenue, Columbus, Ohio 43210, USA.
SOURCE:             METABOLIC ENGINEERING, (2001 Jan) 3 (1) 49-63.
                    Journal code: 9815657. ISSN: 1096-7176.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200105
ENTRY DATE:         Entered STN: 20010604
                    Last Updated on STN: 20010604
                    Entered Medline: 20010531
AB   The biosynthesis of daunorubicin and its precursors proceeds via the
     condensation of nine C-2 units derived from malonyl-CoA onto a propionyl
     starter moiety. The daunorubicin polyketide biosynthesis gene cluster of
     Streptomyces sp. strain C5 has two unique open reading frames, dpsC and
     dpsD, encoding, respectively, a fatty acid ketoacyl synthase (KAS) III
     homologue that is lacking an active-site cysteine and a proposed acyl-CoA:
     acyl carrier protein acyltransferase. The two
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genes are positioned directly downstream of dpsA and dpsB which encode the alpha and beta components of the type II KAS, respectively. Expression of the dpsABCDEFGdauGI genes in Streptomyces lividans resulted in the formation of aklanonic acid, the first stable **chromophore** of the daunorubicin biosynthesis pathway. Deletion of dpsC, but not dpsD, from this gene set resulted in the formation of desmethylaklanonic acid, derived from an acetyl-CoA starter unit, and aklanonic acid, derived from propionyl-CoA, in a 60:40 ratio. Thus, DpsC contributes to the selection of propionyl-CoA as the starter unit but does not alone dictate it. A dpsCD deletion mutant of Streptomyces sp. strain C5 (C5VR5) still produced daunorubicin but, more significantly, anthracycline and anthracyclinone derivatives resulting from the use of acetyl-CoA as an alternative starter moiety. Expression of dpsC, but not dpsD, in mutant C5VR5 restored the wild-type phenotype. Among the new compounds was the new biosynthesis product feudomycin D. These results suggest that in the absence of DpsC, the daunorubicin PKS complex behaves promiscuously, utilizing both acetyl-CoA (ca. 60% of the time) and propionyl-CoA (ca. 40%) as starter units. The fact that DpsC is not required for initiation with propionyl-CoA is significant, as the information must then lie in other components of the PKS complex. We propose to call DpsC the propionyl starter unit "fidelity factor."

L4 ANSWER 2 OF 5 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1999425106 MEDLINE
DOCUMENT NUMBER: 99425106 PubMed ID: 10493786
TITLE: Mossbauer studies of the formation and reactivity of a quasi-stable peroxo intermediate of stearoyl-**acyl carrier protein** Delta 9-desaturase.
AUTHOR: Broadwater J A; Achim C; Munck E; Fox B G
CORPORATE SOURCE: The Institute for Enzyme Research, Graduate School, University of Wisconsin, Madison 53705, USA.
CONTRACT NUMBER: GM-22701 (NIGMS)
GM-50853 (NIGMS)
T32 GM-08293 (NIGMS)
SOURCE: BIOCHEMISTRY, (1999 Sep 21) 38 (38) 12197-204.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991028

AB Stearoyl-ACP Delta(9)-desaturase (Delta 9D) is a diiron enzyme that catalyzes 18:0-ACP desaturation. Each subunit of homodimeric resting Delta 9D contains a diferric cluster, while chemical reduction by 4e(-) produces a diferrous cluster in each subunit. Reaction of 4e(-)-reduced Delta 9D with 18:0-ACP and O(2) yields a blue **chromophore** (lambda(max) approximately 700 nm) that exhibits a vibrational spectrum indicative of a micro-1,2-peroxo complex; this species has been designated peroxo Delta9D. In contrast to other enzymic peroxodiiron intermediates, peroxo Delta 9D is long-lived (t(1/2) approximately 30 min at 25 degrees C) and decays via an oxidase reaction without formation of either H(2)O(2) or product (18:1-ACP). In this work, optical, transient kinetic, and Mossbauer techniques have been used to further investigate the origin and nature of this unusual peroxodiiron complex. Rapid mixing of 4e(-) Delta 9D with O(2)-equilibrated 18:0-ACP produced peroxo Delta 9D as revealed by a temperature-dependent, pseudo-first-order absorption increase at 700 nm (k = 46 s(-)(1) at 6 degrees C). The Mossbauer spectrum of peroxo Delta 9D, accounting for 96% of the total iron, consists of two quadrupole doublets present in equal proportions: delta(1) = 0.68(1) mm/s, and Delta E(Q)(1) = 1.90(2) mm/s; delta(2) = 0.64(1) mm/s, and Delta E(Q)(2) = 1.06(2) mm/s. Decay of the 700 nm optical band (k = 0.004 min(-)(1) at 6 degrees C) correlates with the complete conversion of peroxo Delta 9D into

a complex called peroxo-cycled Delta 9D, which exhibits two new doublets present in equal proportions: delta(1) = 0.57(2) mm/s, and Delta E(Q)(1) = 1. 91(3) mm/s; delta(2) = 0.52(2) mm/s, and Delta E(Q)(2) = 1.41(3) mm/s. Thus, peroxo Delta 9D contains two asymmetric diferric clusters and reacts to yield peroxo-cycled Delta 9D, also containing two asymmetric diferric clusters that most probably represent a substrate complex state. The clusters of both peroxo Delta 9D and peroxo-cycled Delta 9D have a diamagnetic ground state. Because peroxo Delta 9D and peroxo-cycled Delta 9D are observed only in the presence of 18:0-ACP, substrate binding appears to have introduced asymmetry into the Delta 9D diiron clusters. In situ photolysis of peroxo Delta 9D at 4.2 K in the Mossbauer cryostat caused the release of O(2) and the reappearance of a diferrous Delta 9D.18:0-ACP complex with slightly changed parameters, suggesting a constrained cluster configuration was produced by the photolysis event. Annealing the photolyzed sample for 30 min at 77 K quantitatively restored the Mossbauer spectrum of peroxo Delta 9D, showing that the released O(2) was effectively sequestered within the active site.

L4 ANSWER 3 OF 5 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1999238302 MEDLINE
DOCUMENT NUMBER: 99238302 PubMed ID: 10220314
TITLE: The ferroxidase reaction of ferritin reveals a diferric mu-1,2 bridging peroxide intermediate in common with other O2-activating non-heme diiron proteins.
AUTHOR: Moenne-Loccoz P; Krebs C; Herlihy K; Edmondson D E; Theil E C; Huynh B H; Loehr T M
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Oregon Graduate Institute of Science and Technology, Portland 97291-1000, USA.. ploccoz@bmb.ogi.edu
CONTRACT NUMBER: DK-20251 (NIDDK)
GM-18865 (NIGMS)
GM-47295 (NIGMS)
SOURCE: BIOCHEMISTRY, (1999 Apr 27) 38 (17) 5290-5.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 19990601
Last Updated on STN: 20021210
Entered Medline: 19990514

AB Ferritins are ubiquitous proteins that concentrate, store, and detoxify intracellular iron through oxidation of Fe2+ (ferroxidation), followed by translocation and hydrolysis to form a large inorganic mineral core. A series of mutagenesis, kinetics, and spectroscopic studies of ferritin led to the proposal that the oxidation/translocation path involves a diiron protein site. Recent stopped-flow absorption and rapid freeze-quench Mossbauer studies have identified a single peroxodiferric species as the initial transient intermediate formed in recombinant frog M ferritin during rapid ferroxidation [Pereira, S. A., Small, W., Krebs, C., Tavares, P., Edmondson, D. E., Theil, E. C., and Huynh, B. H. (1998) Biochemistry 37, 9871-9876]. To further characterize this transient intermediate and to establish unambiguously the peroxodiferric assignment, rapid freeze-quenching was used to trap the initial intermediate for resonance Raman investigation. Discrete vibrational modes are observed for this intermediate, indicating a single **chromophore** in a homogeneous state, in agreement with the Mossbauer conclusions. The frequency at 851 cm-1 is assigned as nu(O-O) of the bound peroxide, and the pair of frequencies at 485 and 499 cm-1 is attributed, respectively, to nus and nuas of Fe-O2-Fe. Identification of the **chromophore** as a micro-1,2 bridged diferric peroxide is provided by the isotope sensitivity of these Raman bands. Similar peroxodiferric intermediates have been detected in a mutant of the R2 subunit of ribonucleotide reductase from Escherichia coli and chemically reduced Delta9 stearoyl-

acyl carrier protein desaturase (Delta9D), but in contrast, the ferritin intermediate is trapped from the true reaction pathway of the native protein. Differences in the Raman signatures of these peroxide species are assigned to variations in Fe-O-O-Fe angles and may relate to whether the iron is retained in the catalytic center or released as an oxidized product.

L4 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:219684 CAPLUS
DOCUMENT NUMBER: 131:111909
TITLE: Biosynthetic gene clusters of benzoisochromanequinone antibiotics in Streptomyces spp. Identification of genes involved in post-PKS tailoring steps
AUTHOR(S): Ichinose, Koji; Taguchi, Takaaki; Ebizuka, Yutaka; Hopwood, David A.
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: Actinomycetologica (1998), 12(2), 99-109
CODEN: ACTIF4; ISSN: 0914-5818
PUBLISHER: Society for Actinomycetes Japan
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 64 refs. Benzoisochromanequinone (BIQ) antibiotics are a class of arom. polyketides produced by Streptomyces spp. A polyketide synthase (PKS, Type II) is involved in the formation of each BIQ **chromophore**. The first PKS genes were cloned in S. coelicolor A3(2), producer of the typical BIQ antibiotic, actinorhodin; sequence anal. of the PKS genes revealed their clustering with all the other relevant biosynthetic genes (the act cluster). A no. of PKS genes have been discovered in the producers not only of other BIQs, but also of other classes of arom. polyketides, using the act PKS genes as hybridization probes. Among them similar genetic organizations were identified for the genes encoding the minimal PKS components - KS (ketosynthase), CLF (chain length factor), ACP (**acyl carrier protein**) - and their closely assocd. proteins: KR (ketoreductase), ARO (aromatase), and CYC (cyclase). In spite of the increasing knowledge of PKS genes themselves, various biosynthetic problems remain to be solved. As one of the most extensively studied examples at the genetic level, BIQ antibiotics are useful model compds. to be studied to understand the whole biosynthetic pathways of arom. polyketides. This review describes the genes from the act and gra (granaticin biosynthetic gene cluster in S. violaceoruber Tu22) clusters involved in post-PKS modifying (tailoring) steps to complete BIQ biosynthesis.
REFERENCE COUNT: 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 5 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 83307387 MEDLINE
DOCUMENT NUMBER: 83307387 PubMed ID: 6614912
TITLE: Environment of the aromatic chromophores of **acyl carrier protein**.
AUTHOR: Rock C O
CONTRACT NUMBER: CA21765 (NCI)
GM29053 (NIGMS)
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1983 Aug) 225 (1) 122-9.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198310
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19831008

AB **Acyl carrier protein** contains two phenylalanines (residues 28 and 50) and one tyrosine (residue 71). The environment of these chromophores was assessed using first-derivative spectroscopy to examine the uv absorption spectrum of **acyl carrier protein** in detail. In particular, the phenylalanine absorption maxima were perturbed from the water spectrum, and experiments with model systems suggested that the phenylalanines of **acyl carrier protein** reside in an environment more similar to acetonitrile than water. The spectrum in the phenylalanine region resulted from the tertiary folding of the protein since these features disappeared in the absorption spectrum of the denatured **acyl carrier protein**. Tyrosine-71 appears to be a partially buried residue based on the native minus denatured ACP difference spectrum as well as solvent and thermal perturbation spectra. The attachment of a fatty acid to **acyl carrier protein** resulted in a shift in the absorption spectrum of tyrosine-71 consistent with this **chromophore** being in a more hydrophobic environment in the acylated protein. The apolar environment of the aromatic amino acids in **acyl carrier protein** suggests that they are structural components of the hydrophobic sequences that comprise the fatty acid-binding domain of this protein.

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=> s acyl carrier protein and chemical modification
L5          35 ACYL CARRIER PROTEIN AND CHEMICAL MODIFICATION

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6          19 DUP REM L5 (16 DUPLICATES REMOVED)

=> focus l6
PROCESSING COMPLETED FOR L6
L7          19 FOCUS L6 1-

=> d l7 1-10 ibib ab

L7   ANSWER 1 OF 19  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER:   2000:88184  BIOSIS
DOCUMENT NUMBER:    PREV200000088184
TITLE:              Crystallization of the NADP-dependent beta-keto
                    acyl-carrier protein reductase
                    from Brassica napus.
AUTHOR(S):          Fisher, Martin; Sedelnikova, Svetlana E.; Martindale,
                    Wayne; Thomas, Neil C.; Simon, J. William; Slabas, Antoni
                    R.; Rafferty, John B. [Reprint author]
CORPORATE SOURCE:   Department of Molecular Biology and Biotechnology, Krebs
                    Institute for Biomolecular Research, University of
                    Sheffield, Sheffield, S10 2TN, UK
SOURCE:             Acta Crystallographica Section D Biological
                    Crystallography, (Jan., 2000) Vol. 56, No. 1, pp. 86-88.
                    print.
                    ISSN: 0907-4449.
DOCUMENT TYPE:      Article
LANGUAGE:           English
ENTRY DATE:         Entered STN: 10 Mar 2000
                    Last Updated on STN: 3 Jan 2002
AB   The NADP-dependent beta-keto acyl-carrier
     protein reductase (BKR) from Brassica napus has been crystallized
     by the hanging-drop vapour-diffusion method using polyethylene glycol of
     average molecular weight 1500 as the precipitant. The crystals belong to
     the hexagonal space group P6422, with unit-cell parameters a = b = 129.9,
     c = 93.1 114, alpha = beta = 90, gamma = 120degree. Calculated values for
     Vm, the use of rotation and translation functions and consideration of the
     packing suggest that the asymmetric unit contains a monomer. The crystals
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diffract to beyond 2.8 ANG resolution and are more amenable to X-ray diffraction analysis than those reported previously for the Escherichia coli enzyme. The structure determination of B. napus BKR will provide important insights into the catalytic mechanism of the enzyme and into the evolution of the fatty-acid elongation cycle by comparisons with the other oxidoreductase of the pathway, enoyl **acyl-carrier protein** reductase (ENR).

L7 ANSWER 2 OF 19 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2003460203 EMBASE
TITLE: Biochemical Characterization of **Acyl Carrier Protein** (AcpM) and Malonyl-CoA:AcpM Transacylase (mtFabD), Two Major Components of Mycobacterium tuberculosis Fatty Acid Synthase II.
AUTHOR: Kremer L.; Nampoothiri K.M.; Lesjean S.; Dover L.G.; Graham S.; Betts J.; Brennan P.J.; Minnikin D.E.; Locht C.; Besra G.S.
CORPORATE SOURCE: G.S. Besra, Dept. of Microbiology and Immunology, University of Newcastle, Newcastle upon Tyne NE2 4HH, France. g.s.besra@newcastle.ac.uk
SOURCE: Journal of Biological Chemistry, (27 Jul 2001) 276/30 (27967-27974).
Refs: 43
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Malonyl coenzyme A (CoA)-**acyl carrier protein** (ACP) transacylase (MCAT) is an essential enzyme in the biosynthesis of fatty acids in all bacteria, including Mycobacterium tuberculosis. MCAT catalyzes the transacylation of malonate from malonyl-CoA to activated holo-ACP, to generate malonyl-ACP, which is an elongation substrate in fatty acid biosynthesis. To clarify the roles of the mycobacterial **acyl carrier protein** (AcpM) and MCAT in fatty acid and mycolic acid biosynthesis, we have cloned, expressed, and purified acpM and mtfabD (malonyl-CoA: AcpM transacylase) from M. tuberculosis. According to the culture conditions used, AcpM was produced in Escherichia coli in two or three different forms: apo-AcpM, holo-AcpM, and palmitoylated-AcpM, as revealed by electrospray mass spectrometry. The mtfabD gene encoding a putative MCAT was used to complement a thermosensitive E. coli fabD mutant. Expression and purification of mtFabD resulted in an active enzyme displaying strong MCAT activity in vitro. Enzymatic studies using different ACP substrates established that holo-AcpM constitutes the preferred substrate for mtFabD. In order to provide further insight into the structure-function relationship of mtFabD, different mutant proteins were generated. All mutations (Q9A, R116A, H194A, Q243A, S91T, and S91A) completely abrogated MCAT activity in vitro, thus underlining the importance of these residues in transacylation. The generation and characterization of the AcpM forms and mtFabD opens the way for further studies relating to fatty acid and mycolic acid biosynthesis to be explored in M. tuberculosis. Since a specific type of FabD is found in mycobacterial species, it represents an attractive new drug target waiting to be exploited.

L7 ANSWER 3 OF 19 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2001346296 EMBASE
TITLE: An unusual .beta.-ketoacyl:**acyl carrier protein** synthase and acyltransferase motifs in taK, a putative protein required for biosynthesis of the antibiotic TA in Myxococcus xanthus.

AUTHOR: Paitan Y.; Orr E.; Ron E.Z.; Rosenberg E.
CORPORATE SOURCE: E. Rosenberg, Department of Molecular Microbiology, George S. Wise Faculty of Life Sci., Tel Aviv University, Ramat Aviv, Israel. eliora@post.tau.ac.il
SOURCE: FEMS Microbiology Letters, (25 Sep 2001) 203/2 (191-197). Refs: 42
ISSN: 0378-1097 CODEN: FMLED7
PUBLISHER IDENT.: S 0378-1097(01)00350-0
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The antibiotic TA of Myxococcus xanthus is produced by a type-I polyketide synthase mechanism. Previous studies have indicated that TA genes are clustered within a 36-kb region. The chemical structure of TA indicates the need for several post-modification steps, which are introduced to form the final bioactive molecule. These include three C-methylations, an O-methylation and a specific hydroxylation. In this study, we describe the genetic analysis of taK, encoding a specific polyketide .beta.-ketoacyl: **acyl carrier protein** synthase, which contains an unusual .beta.-ketoacyl synthase and acyltransferase motifs and is likely to be involved in antibiotic TA post-modification. Functional analysis of this .beta.-ketoacyl:**acyl carrier protein** synthase by specific gene disruption suggests that it is essential for the production of an active TA molecule.

L7 ANSWER 4 OF 19 MEDLINE on STN
ACCESSION NUMBER: 75114818 MEDLINE
DOCUMENT NUMBER: 75114818 PubMed ID: 234965
TITLE: On the structure-function relationship of **acyl carrier protein** of Escherichia coli.
AUTHOR: Schulz H
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1975 Mar 25) 250 (6) 2299-304.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197506
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19970203
Entered Medline: 19750606

AB The conformations of Escherichia coli **acyl carrier protein** (ACP) and acetylated ACP have been studied as a function of pH and salt concentration by circular dichroism measurements. The results show that the amino groups of ACP in their protonated form are important for maintaining the native conformation of the protein at physiological pH. However, externally added cations (divalent more effectively than monovalent ones) can substitute for the ammonium groups in maintaining the ordered structure pf ACP. It is suggested that both the ammonium groups of ACP and externally added cations reduce the repulsion between carboxylate groups of ACP and thereby prevent the unfolding of the protein. A reduction of the number of negatively charged carboxylate groups by either protonation or **chemical modification** abolished the requirement for either ammonium groups or other cations. A qualitative agreement between the effect of salt on the conformation and on the biological activity of acetylated ACP has been observed. The single arginine residue of acetylated ACP has been modified by treatment with a trimer of 2,3-butanedione with the resulting derivative of ACP retaining most of its biological activity.

L7 ANSWER 5 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:48362 BIOSIS
DOCUMENT NUMBER: PREV200100048362
TITLE: Chemical and posttranslational modification of Escherichia coli **acyl carrier protein** for preparation of dansyl-acyl carrier proteins.
AUTHOR(S): Haas, Jeffrey A.; Frederick, Melissa A.; Fox, Brian G. [Reprint author]
CORPORATE SOURCE: Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI, 53706, USA
bgfox@biochem.wisc.edu
SOURCE: Protein Expression and Purification, (November, 2000) Vol. 20, No. 2, pp. 274-284. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jan 2001
Last Updated on STN: 12 Feb 2002

AB Escherichia coli **acyl carrier protein** (ACP) contains a single tyrosine residue at position 71. The combined o-nitration of apo-ACP Y71 by tetranitromethane and reduction to 3-aminotyrosyl-apo-ACP were performed to introduce a specific site for attachment of a dansyl fluorescent label. Conditions for purification and characterization of dansylaminotyrosyl-apo-ACP are reported. Dansylaminotyrosyl-apo-ACP was enzymatically phosphopantetheinylated and acylated in vitro with an overall apprx30% yield of purified stearoyl-dansylaminotyrosyl-ACP starting from unmodified apo-ACP. The steady-state kinetic parameters kcat = 22 min-1 and KM = 2.7 muM were determined for reaction of stearoyl-dansylaminotyrosyl-ACP with stearoyl-ACP DELTA9-desaturase. These results show that dansylaminotyrosyl-ACP will function well for studying binding interactions with the DELTA9-desaturase and suggest similar possibilities for other ACP-dependent enzymes. The efficient in vivo phosphopantetheinylation of E. coli apo-ACP by coexpression with holo-ACP synthase in E. coli BL21(DE3) using fructose as the carbon source is also reported.

L7 ANSWER 6 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:395682 BIOSIS
DOCUMENT NUMBER: PREV200100395682
TITLE: Crystal structure of the Mycobacterium tuberculosis beta-ketoacyl-**acyl carrier protein** synthase III.
AUTHOR(S): Scarsdale, J. Neel; Kazanina, Galina; He, Xin; Reynolds, Kevin A.; Wright, H. Tonie [Reprint author]
CORPORATE SOURCE: ISBDD, 800 East Leigh St., Suite 212, Virginia Biotechnology Research Park, Richmond, VA, 23219, USA
xrdproc@hsc.vcu.edu
SOURCE: Journal of Biological Chemistry, (June 8, 2001) Vol. 276, No. 23, pp. 20516-20522. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Aug 2001
Last Updated on STN: 22 Feb 2002

AB Mycolic acids (alpha-alkyl-beta-hydroxy long chain fatty acids) cover the surface of mycobacteria, and inhibition of their biosynthesis is an established mechanism of action for several key front-line anti-tuberculosis drugs. In mycobacteria, long chain acyl-CoA products (C14-C26) generated by a type I fatty-acid synthase can be used directly for the alpha-branch of mycolic acid or can be extended by a type II fatty-acid synthase to make the meromycolic acid (C50-C56))-derived component. An unusual Mycobacterium tuberculosis beta-ketoacyl-**acyl carrier protein** (ACP) synthase III

(mtFabH) has been identified, purified, and shown to catalyze a Claisen-type condensation between long chain acyl-CoA substrates such as myristoyl-CoA (C14) and malonyl-ACP. This enzyme, presumed to play a key role in initiating meromycolic acid biosynthesis, was crystallized, and its structure was determined at 2.1-ANG resolution. The mtFabH homodimer is closely similar in topology and active-site structure to Escherichia coli FabH (ecFabH), with a CoA/malonyl-ACP-binding channel leading from the enzyme surface to the buried active-site cysteine residue. Unlike ecFabH, mtFabH contains a second hydrophobic channel leading from the active site. In the ecFabH structure, this channel is blocked by a phenylalanine residue, which constrains specificity to acetyl-CoA, whereas in mtFabH, this residue is a threonine, which permits binding of longer acyl chains. This same channel in mtFabH is capped by an alpha-helix formed adjacent to a 4-amino acid sequence insertion, which limits bound acyl chain length to 16 carbons. These observations offer a molecular basis for understanding the unusual substrate specificity of mtFabH and its probable role in regulating the biosynthesis of the two different length acyl chains required for generation of mycolic acids. This mtFabH presents a new target for structure-based design of novel antimycobacterial agents.

L7 ANSWER 7 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1999:231838 BIOSIS
DOCUMENT NUMBER: PREV199900231838
TITLE: Dibromopropanone cross-linking of the phosphopantetheine and active-site cysteine thiols of the animal fatty acid synthase can occur both inter- and intrasubunit. Reevaluation of the side-by-side, antiparallel subunit model.
AUTHOR(S): Witkowski, Andrzej; Joshi, Anil K.; Rangan, Vangipuram S.; Falick, Arnold M.; Witkowska, H. Ewa; Smith, Stuart [Reprint author]
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, 747 Fifty-second St., Oakland, CA, 94609, USA
SOURCE: Journal of Biological Chemistry, (April 23, 1999) Vol. 274, No. 17, pp. 11557-11563. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 17 Jun 1999
Last Updated on STN: 17 Jun 1999

AB The objective of this study was to test a new model for the homodimeric animal FAS which implies that the condensation reaction can be catalyzed by the amino-terminal beta-ketoacyl synthase domain in cooperation with the penultimate carboxyl-terminal **acyl carrier protein** domain of either subunit. Treatment of animal fatty acid synthase dimers with dibromopropanone generates three new molecular species with decreased electrophoretic mobilities; none of these species are formed by fatty acid synthase mutant dimers lacking either the active-site cysteine of the beta-ketoacyl synthase domain (C161A) or the phosphopantetheine thiol of the **acyl carrier protein** domain (S2151A). A double affinity-labeling strategy was used to isolate dimers that carried one or both mutations on one or both subunits; the heterodimers were treated with dibromopropanone and analyzed by a combination of sodium dodecyl sulfate/polyacrylamide gel electrophoresis, Western blotting, gel filtration, and matrix-assisted laser desorption mass spectrometry. Thus the two slowest moving of these species, which accounted for 45 and 15% of the total, were identified as doubly and singly cross-linked dimers, respectively, whereas the fastest moving species, which accounted for 35% of the total, was identified as originating from internally cross-linked subunits. These results show that the two polypeptides of the fatty acid synthase are oriented such that head-to-tail contacts are formed both between and within subunits, and provide the first structural evidence in support of the new model.

L7 ANSWER 8 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:114010 BIOSIS
DOCUMENT NUMBER: PREV199698686145
TITLE: Transacylase-like structure and its role in substrate channeling of 6-hydroxymellein synthase, a multifunctional polyketide biosynthetic enzyme in carrot cell extracts.
AUTHOR(S): Kurosaki, Fumiya
CORPORATE SOURCE: Cell Biol. Lab., Fac. Pharmaceutical Sci., Toyama Med. Pharmaceutical Univ., Sugitani, Toyama 930-01, Japan
SOURCE: FEBS Letters, (1996) Vol. 379, No. 1, pp. 97-102. CODEN: FEBLAL. ISSN: 0014-5793.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Mar 1996
Last Updated on STN: 10 Jun 1997

AB 6-Hydroxymellein synthase, a multifunctional polyketide biosynthetic enzyme of carrot, lost the binding ability toward its co-substrates, acetyl- and malonyl-CoAs, by the treatment with the blocking reagents for serine-OH. In contrast, the enzyme retained the binding ability even when the two SH groups at the reaction center (cysteine-SH of the condensation enzyme and cysteamine-SH of **acyl carrier protein**) were blocked, and one substrate bound to the SH-blocked enzyme was readily replaced by the other. It appeared that the cysteine-SH accepted only acetyl moiety while cysteamine-SH was preferentially malonylated in the presence of both of the substrates. These results suggest that transacylase-like domain is involved in the structure of 6-hydroxymellein synthase as a common primary binding site of its co-substrates, and acetyl and malonyl moieties are properly channeled from their CoA esters to cysteine-SH and **acyl carrier protein**-SH via this domain, respectively.

L7 ANSWER 9 OF 19 MEDLINE on STN
ACCESSION NUMBER: 1999178852 MEDLINE
DOCUMENT NUMBER: 99178852 PubMed ID: 10079090
TITLE: HlyC, the internal protein acyltransferase that activates hemolysin toxin: role of conserved histidine, serine, and cysteine residues in enzymatic activity as probed by **chemical modification** and site-directed mutagenesis.
AUTHOR: Trent M S; Worsham L M; Ernst-Fonberg M L
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City 37614, USA.
CONTRACT NUMBER: GM/OD54337 (NIGMS)
SOURCE: BIOCHEMISTRY, (1999 Mar 16) 38 (11) 3433-9. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990426
Last Updated on STN: 20020420
Entered Medline: 19990415

AB HlyC is an internal protein acyltransferase that activates hemolysin, a toxic protein produced by pathogenic Escherichia coli. Acyl-**acyl carrier protein** (ACP) is the essential acyl donor. Separately subcloned, expressed, and purified prohemolysin A (proHlyA), HlyC, and [1-14C]myristoyl-ACP have been used to study the conversion of proHlyA to HlyA [Trent, M. S., Worsham, L. M., and Ernst-Fonberg, M. L. (1998) Biochemistry 37, 4644-4655]. HlyC and hemolysin belong to a family of at least 13 toxins produced by Gram-negative bacteria. The homologous acyltransferases of the family show a number of conserved residues that are possible candidates for participation in acyl transfer. Specific chemical reagents and site-directed mutagenesis showed that neither the

single conserved cysteine nor the three conserved serine residues were required for enzyme activity. Treatment with the reversible histidine-modifying diethyl pyrocarbonate (DEPC) inhibited acyltransferase activity, and acyltransferase activity was restored following hydroxylamine treatment. The substrate myristoyl-ACP protected HlyC from DEPC inhibition. These findings and spectral absorbance changes suggested that histidine, particularly a histidine proximal to the substrate binding site, was essential for enzyme activity. Site-directed mutageneses of the single conserved histidine residue, His23, to alanine, cysteine, or serine resulted in each instance in complete inactivation of the enzyme.

L7 ANSWER 10 OF 19 MEDLINE on STN
ACCESSION NUMBER: 1999410444 MEDLINE
DOCUMENT NUMBER: 99410444 PubMed ID: 10480918
TITLE: The active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase. **Chemical modification** and site-directed mutagenesis.
AUTHOR: Wyckoff T J; Raetz C R
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710, USA.
CONTRACT NUMBER: GM-51310 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Sep 17) 274 (38) 27047-55.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991013

AB UDP-N-acetylglucosamine (UDP-GlcNAc) acyltransferase (LpxA) catalyzes the reversible transfer of an R-3-hydroxyacyl chain from R-3-hydroxyacyl-**acyl carrier protein** to the glucosamine 3-OH of UDP-GlcNAc in the first step of lipid A biosynthesis. Lipid A is required for the growth and virulence of most Gram-negative bacteria, making its biosynthetic enzymes intriguing targets for the development of new antibacterial agents. LpxA is a member of a large family of left-handed beta-helical proteins, many of which are acyl- or acetyltransferases. We now demonstrate that histidine-, lysine-, and arginine-specific reagents effectively inhibit LpxA of Escherichia coli, whereas serine- and cysteine-specific reagents do not. Using this information in conjunction with multiple sequence alignments, we constructed site-directed alanine substitution mutations of conserved histidine, lysine, and arginine residues. Many of these mutant LpxA enzymes show severely decreased specific activities under standard assay conditions. The decrease in activity corresponds to decreased k(cat)/K(m,UDP-GlcNAc) values for all the mutants. With the exception of H125A, in which no activity is seen under any assay condition, the decrease in k(cat)/K(m,UDP-GlcNAc) mainly reflects an increased K(m,UDP-GlcNAc). His(125) of E. coli LpxA may therefore function as a catalytic residue, possibly as a general base. LpxA does not catalyze measurable UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc hydrolysis or UDP-GlcNAc/UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc exchange, arguing against a ping-pong mechanism with an acyl-enzyme intermediate.

=> s acyl carrier protein and apo-acyl carrier protein and holo-acyl carrier protein and
L8 0 ACYL CARRIER PROTEIN AND APO-ACYL CARRIER PROTEIN AND HOLO-ACYL CARRIER PROTEIN AND ACYLATED ACYL CARRIER PROTEIN

=> s (acyl carrier protein or apo-acyl carrier protein or holo-acyl carrier protein or a
L9 79 (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACYL CARRIER PROTEIN OR ACYLATED ACYL CARRIER PROTEIN) AND LABEL

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10 41 DUP REM L9 (38 DUPLICATES REMOVED)

=> d l7 11-19 ibib ab

L7 ANSWER 11 OF 19 MEDLINE on STN
ACCESSION NUMBER: 1999321521 MEDLINE
DOCUMENT NUMBER: 99321521 PubMed ID: 10393560
TITLE: HlyC, the internal protein acyltransferase that activates hemolysin toxin: the role of conserved tyrosine and arginine residues in enzymatic activity as probed by **chemical modification** and site-directed mutagenesis.
AUTHOR: Trent M S; Worsham L M; Ernst-Fonberg M L
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City 37614, USA.
CONTRACT NUMBER: GM/OD54337 (NIGMS)
SOURCE: BIOCHEMISTRY, (1999 Jul 6) 38 (27) 8831-8.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990816
Last Updated on STN: 20020420
Entered Medline: 19990802

AB Internal fatty acylation of proteins is a recognized means of modifying biological behavior. Escherichia coli hemolysin A (HlyA), a toxic protein, is transcribed as a nontoxic protein and made toxic by internal acylation of two lysine residue epsilon-amino groups; HlyC catalyzes the acyl transfer from acyl-**acyl carrier protein** (ACP), the obligate acyl donor. Conserved residues among the respective homologous C proteins that activate 13 different RTX (repeats in toxin) toxins of which HlyA is the prototype likely include some residues that are important in catalysis. Possible roles of two conserved tyrosines and two conserved arginines were investigated by noting the effects of chemical modifiers and site-directed mutagenesis. TNM modification of HlyC at pH 8.0 led to extensive inhibition that was prevented by the presence of the substrate myristoyl-ACP but not by the product, ACPSH. NAI had no effect. Y70G and Y150G greatly diminished enzyme activity, whereas mutations Y70F and Y150F exhibited wild-type activity. Modification of arginine residues with PG markedly lowered acyltransferase activity with moderate protection by both myristoyl-ACP and ACPSH. Under optimum conditions, four separate mutations of the two conserved arginine residues (R24A, R24K, R87A, and R87K) had little effect on acyltransferase activity.

L7 ANSWER 12 OF 19 MEDLINE on STN
ACCESSION NUMBER: 94165062 MEDLINE
DOCUMENT NUMBER: 94165062 PubMed ID: 8120025
TITLE: An essential histidine residue required for fatty acylation and acyl transfer by myristoyltransferase from luminescent bacteria.
AUTHOR: Ferri S R; Meighen E A
CORPORATE SOURCE: Department of Biochemistry, McGill University, Montreal, Quebec, Canada.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Mar 4) 269 (9) 6683-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940412
Last Updated on STN: 19970203
Entered Medline: 19940404

AB The lux-specific acyltransferases are serine esterases responsible for preferential diversion of myristic acid from fatty acid biosynthesis to the luminescent system. In contrast to other acyltransferases, an acylated enzyme intermediate can readily be detected making it ideal for the study of the mechanism of acyl transfer. Although the transferase readily cleaves **acyl carrier protein** and acyl-CoA, an alternate more rapid and convenient assay involving the cleavage of p-nitrophenyl acyl esters was developed and applied in these studies. The cleavage of the oxyesters by the transferase was shown to have a similar dependence on fatty acid chain length and organic solvents as the cleavage of thioesters. Using this assay, it could be demonstrated that the Photobacterium phosphoreum transferase was inactivated at pH 6 with diethyl pyrocarbonate at a rate (73 M-1 s-1, 10 degrees C) even faster than that reported for other enzymes with reactive histidyl residues at their active site. Spectral changes during **chemical modification** as well as restoration of activity by neutral hydroxylamine showed that the loss of activity was associated with modification of a single histidine residue. Replacement of the four histidine residues, conserved in all lux-specific acyltransferases, by asparagine demonstrated that cleavage of both thioesters and oxyesters by the P. phosphoreum acyltransferase as well as acylation of the enzyme was blocked on mutation of His-244 but not the other three conserved histidines (His-12, -52, and -75). These results suggest that the histidine residue near the carboxyl terminus (His-244) may be part of a catalytic triad essential for cleavage of acyl esters and transfer of the acyl group to the enzyme.

L7 ANSWER 13 OF 19 EMBASE
on STN

ACCESSION NUMBER: 2001036235 EMBASE
TITLE: Purification, priming, and catalytic acylation of carrier protein domains in the polyketide synthase and nonribosomal peptidyl synthetase modules of the HMWP1 subunit of yersiniabactin synthetase.
AUTHOR: Suo Z.; Tseng C.C.; Walsh C.T.
CORPORATE SOURCE: C.T. Walsh, Dept. Biol. Chem./Molec. Pharmacol., Harvard Medical School, Boston, MA 02115, United States. walsh@walsh.med.harvard.edu
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2 Jan 2001) 98/1 (99-104). Refs: 24
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The 207-kDa polyketide synthase (PKS) module (residues 1-1895) and the 143-kDa nonribosomal peptidyl synthetase (NRPS) module (1896-3163) of the 350-kDa HMWP1 subunit of yersiniabactin synthetase have been expressed in and purified from Escherichia coli in soluble forms to characterize the **acyl carrier protein** (ACP) domain of the PKS module and the homologous peptidyl carrier protein (PCP(3)) domain of the NRPS module. The apo-ACP and PCP domains could be selectively posttranslationally primed by the E. coli ACPS and EntD phosphopantetheinyl transferases (PPTases), respectively, whereas the Bacillus subtilis PPTase Sfp primed both carrier protein domains in vitro or during in vivo coexpression. The holo-NRPS module but not the holo-PKS module was then selectively aminoacylated with cysteine by the adenylation

domain embedded in the HMWP2 subunit of yersiniabactin synthetase, acting in trans. When the acyltransferase (AT) domain of HMWP1 was analyzed for its ability to malonylate the holo carrier protein domains, in cis acylation was first detected. Then, in trans malonylation of the excised holo-ACP or holo-PCP(3)-TE fragments by HMWP1 showed both were malonylated with a 3:1 catalytic efficiency ratio, showing a promiscuity to the AT domain.

L7 ANSWER 14 OF 19 MEDLINE on STN
ACCESSION NUMBER: 81044631 MEDLINE
DOCUMENT NUMBER: 81044631 PubMed ID: 6775990
TITLE: Selective **chemical modification** of the active sites of the ketoacyl reductase and enoyl reductase of fatty acid synthetase from lactating rat mammary glands.
AUTHOR: Poulose A J; Rogers L; Kolattukudy P E
CONTRACT NUMBER: GM-18278 (NIGMS)
SOURCE: INTERNATIONAL JOURNAL OF BIOCHEMISTRY, (1980) 12 (4) 591-6. Journal code: 0250365. ISSN: 0020-711X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198101
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19810116

L7 ANSWER 15 OF 19 EMBASE on STN
ACCESSION NUMBER: 2000000173 EMBASE
TITLE: An ordered reaction mechanism for bacterial toxin acylation by the specialized acyltransferase HlyC: Formation of a ternary complex with acylACP and protoxin substrates.
AUTHOR: Stanley P.; Hyland C.; Koronakis V.; Hughes C.
CORPORATE SOURCE: P. Stanley, Department of Structural Medicine, Cambridge Inst. for Medical Research, Wellcome Trust-MRC Building, Hills Road, Cambridge CB2 2XY, United Kingdom. plds1@mole.bio.cam.ac.uk
SOURCE: Molecular Microbiology, (1999) 34/5 (887-901). Refs: 56
ISSN: 0950-382X CODEN: MOMIEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
052 Toxicology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The 110 kDa haemolysin protoxin (proHlyA) is activated in the Escherichia coli cytosol by **acyl carrier protein** -dependent fatty acylation of two internal lysine residues, directed by the co-synthesized protein HlyC. Using an in vitro maturation reaction containing purified protoxin peptides and acylACP, we show unambiguously that HlyC possesses an apparently unique acyltransferase activity fully described by Michaelis-Menten analysis. The V(max) of HlyC at saturating levels of both substrates was .simeq. 115 nmol acyl group min-1 mg-1 with KM(acylACP) of 260 nM and KM(proHlyA) of 27 nM, kinetic parameters sufficient to explain why in vivo HlyC is required at a concentration equimolar to proHlyA. HlyC bound the fatty acyl group from acylACP to generate an acylated HlyC intermediate that was depleted in the presence of proHlyA, but enriched in the presence of proHlyA derivatives lacking acylation target sites. HlyC was also able to bind in vivo 4'-phosphopantetheine. Substitution of conserved amino acids that could act as putative covalent attachment sites did not prevent binding of the fatty acyl or 4'-phosphopantetheine groups. These data and substrate variation analyses suggest that the unique acylation reaction does not

involve covalent attachment of fatty acid to the acyltransferase, but rather that it proceeds via a sequential ordered Bi-Bi reaction mechanism, requiring the formation of a non-covalent ternary acylACP-HlyC-proHlyA complex.

L7 ANSWER 16 OF 19 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:324006 BIOSIS
DOCUMENT NUMBER: PREV199900324006
TITLE: Crystal structure of the Mycobacterium tuberculosis enoyl-ACP reductase, InhA, in complex with NAD+ and a C16 fatty acyl substrate.
AUTHOR(S): Rozwarski, Denise A.; Vilcheze, Catherine; Sugantino, Michele; Bittman, Robert; Sacchettini, James C. [Reprint author]
CORPORATE SOURCE: Department of Biochemistry and Biophysics, Texas A and M University, College Station, TX, 77843-2128, USA
SOURCE: Journal of Biological Chemistry, (May 28, 1999) Vol. 274, No. 22, pp. 15582-15589. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Aug 1999
Last Updated on STN: 24 Aug 1999

AB Enoyl-ACP reductases participate in fatty acid biosynthesis by utilizing NADH to reduce the trans double bond between positions C2 and C3 of a fatty acyl chain linked to the **acyl carrier protein**. The enoyl-ACP reductase from Mycobacterium tuberculosis, known as InhA, is a member of an unusual FAS-II system that prefers longer chain fatty acyl substrates for the purpose of synthesizing mycolic acids, a major component of mycobacterial cell walls. The crystal structure of InhA in complex with NAD+ and a C16 fatty acyl substrate, trans-2-hexadecenoyl-(N-acetylcysteamine)-thioester, reveals that the substrate binds in a general "U-shaped" conformation, with the trans double bond positioned directly adjacent to the nicotinamide ring of NAD+. The side chain of Tyr158 directly interacts with the thioester carbonyl oxygen of the C16 fatty acyl substrate and therefore could help stabilize the enolate intermediate, proposed to form during substrate catalysis. Hydrophobic residues, primarily from the substrate binding loop (residues 196-219), engulf the fatty acyl chain portion of the substrate. The substrate binding loop of InhA is longer than that of other enoyl-ACP reductases and creates a deeper substrate binding crevice, consistent with the ability of InhA to recognize longer chain fatty acyl substrates.

L7 ANSWER 17 OF 19 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:933217 SCISEARCH
THE GENUINE ARTICLE: 493VP
TITLE: Insights into the catalytic mechanism of HlyC, the internal protein acyltransferase that activates Escherichia coli hemolysin toxin
AUTHOR: Worsham L M S; Trent M S; Earls L; Jolly C; Ernst-Fonberg M L (Reprint)
CORPORATE SOURCE: E Tennessee State Univ, Dept Biochem & Mol Biol, James H Quillen Coll Med, Box 70581, Johnson City, TN 37614 USA (Reprint); E Tennessee State Univ, Dept Biochem & Mol Biol, James H Quillen Coll Med, Johnson City, TN 37614 USA
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (13 NOV 2001) Vol. 40, No. 45, pp. 13607-13616.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 45
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Hemolysin, a toxic protein secreted by pathogenic Escherichia coli, is converted from nontoxic prohemolysin, proHlyA, to toxic hemolysin, HlyA, by an internal protein acyltransferase, HlyC. Acyl-**acyl carrier protein** (ACP) is the essential acyl donor. The acyltransferase reaction proceeds through two partial reactions and entails formation of a reactive acyl-HlyC intermediate [Trent, M. S., Worsham, L. M., and Ernst-Fonberg, M. L. (1999) Biochemistry 38, 9541-9548]. The ping pong kinetic mechanism implied by these findings was validated using two different acyl-ACP substrates, thus verifying the independence of the previously demonstrated two partial reactions. Assessments of the stability of the acyl-HlyC intermediate revealed an increased stability at pH 8.6 compared to more acidic pHs. Mutations of a single conserved histidine residue essential for catalysis gave minimal activity when substituted with a tyrosine residue and no activity with a lysine residue. Unlike numerous other His23 mutants, however, the H23K enzyme showed significant acyl-HlyC formation although it was unable to transfer the acyl group from the proposed amide bond intermediate to proHlyA. These findings are compatible with transient formation of acyl-His23 during the course of HlyC catalysis. The effects of several other single site-directed mutations of conserved residues of HlyC on different portions of the reaction progress were examined using a 39 500 kDa fragment of proHlyA which was a more effective substrate than intact proHlyA.

L7 ANSWER 18 OF 19 EMBASE on STN

ACCESSION NUMBER: 2003254163 EMBASE
TITLE: Chain cleavage and sulfoxidation of thiastearoyl-ACP upon reaction with stearoyl-ACP desaturase.
AUTHOR: White R.D.; Fox B.G.
CORPORATE SOURCE: B.G. Fox, Department of Biochemistry, Coll. of Agric. and Life Sciences, University of Wisconsin, Madison, WI 53706-1544, United States. bgfox@biochem.wisc.edu
SOURCE: Biochemistry, (1 Jul 2003) 42/25 (7828-7835).
Refs: 34
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The fatty acid analogues 9- and 10-thiastearate were converted to acyl-ACP derivatives by in vitro enzymatic synthesis and reacted with the reconstituted soluble stearoyl-ACP .DELTA.9 desaturase complex. Electrospray ionization mass spectral analysis of the acyl chains purified from the reaction mixtures showed that 10-thiastearoyl-ACP was converted to the 10-sulfoxide as the sole product. In, the presence of (18)O(2), the sulfoxide oxygen was found to be derived exclusively from O(2). This result confirms the ability of the soluble stearoyl-ACP desaturase to catalyze O atom transfer in the presence of the appropriate substrate analogue. Inhibition studies showed that 10-thiastearoyl-ACP was a mixed-type inhibitor of 18:0-ACP, with an apparent K(I) of .apprx.10 .mu.M. Comparable reactions of the stearoyl-ACP desaturase complex with 9-thiastearoyl-ACP gave the 9-sulfoxide as .apprx.5% of the total products, with the O atom again exclusively derived from O(2). The remaining 95% of the total products arose from an acyl chain cleavage reaction between S-9 and C-10. Matrix-assisted laser desorption ionization time-of-flight mass spectral analysis showed that 9-thiastearoyl-ACP had a mass of 9443 amu while the acyl chain cleavage product had a mass of 9322 amu, corresponding to the calculated mass of 8-mercaptooctanoyl-ACP. Recovery of the acyl chain from the ACP product gave the disulfide of 8-mercaptooctanoate (mass of 349.1 amu), arising from the dimerization of 8-mercaptooctanoate during product workup. Gas chromatography - mass spectral analysis also showed the accumulation of nonanal in sealed reaction vials, accounting for the other product of the acyl chain

cleavage reaction. The reactivity at both the 9 and 10 positions of the thia-substituted acyl-ACPs is consistent with the proximity of both positions to the diiron center oxidant in the enzyme - substrate complex. Moreover, the differential reactivity of the 9- and 10-thiastearoyl-ACPs also suggests position-dependent consequences of the reaction within the .DELTA.9D active site. Mechanisms accounting for both sulfoxidation and acyl cleavage reactions by the stearoyl-ACP .DELTA.9 desaturase are proposed.

L7 ANSWER 19 OF 19 MEDLINE on STN
ACCESSION NUMBER: 86104341 MEDLINE
DOCUMENT NUMBER: 86104341 PubMed ID: 3942750
TITLE: The presence of a histidine residue at or near the NADPH binding site of enoyl reductase domain on the multifunctional fatty acid synthetase of chicken liver.
AUTHOR: Vernon C N; Hsu R Y
CONTRACT NUMBER: AM 13390 (NIADDK)
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1986 Jan 17) 869 (1) 23-8. Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198603
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860306

AB **Chemical modification** of chicken liver fatty acid synthetase with the reagent ethoxyformic anhydride causes inactivation of the palmitate synthetase and enoyl reductase activities of the enzyme complex, but without significant effect on its beta-ketoacyl reductase or beta-ketoacyl dehydratase activity. The second-order rate constant of 0.2 mM-1 X s-1 for loss of synthetase activity is equal to the value for enoyl reductase, indicating that ethoxyformylation destroys the ability of the enzyme to reduce the unsaturated acyl intermediate. The specificity of this reagent for histidine residues is indicated by the appearance of a 240 nm absorption band for ethoxyformic histidine corresponding to the modification of 2.1 residues per enzyme dimer, and by the observation that the modified enzyme is readily reactivated by hydroxylamine. A pK value of 7.1 obtained by studies of the pH rate-profile of inactivation is consistent with that of histidine. Moreover, inactivation by ethoxyformic anhydride is unaffected by reversely blocking essential SH groups of the enzyme with 5,5'-dithiobis(2-nitrobenzoic acid), and therefore does not involve the reaction of these groups. The reaction of tyrosyl groups is excluded by an unchanged absorption at 278 nm. In other experiments, it was shown that inactivation of synthetase is protected by pyridine nucleotide cofactors and nucleotide analogs containing a 2'-phosphate group, and is accompanied by the loss of 2.4 NADPH binding sites. These results implicate the presence of a histidine residue at or near the binding site for 2'-phosphate group of pyridine nucleotide in the enoyl reductase domain of the synthetase.

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(FILE 'HOME' ENTERED AT 11:35:15 ON 15 JAN 2004)

FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT 11:35:53 ON 15 JAN 2004

L1 0 S ACYL CARRIER PROTEIN AND NON-RADIOACTIVE LABEL?
L2 0 S ACYL CARRIER PROTEIN AND CHROMO? LABEL?
L3 17 S ACYL CARRIER PROTEIN AND CHROMOPHORE
L4 5 DUP REM L3 (12 DUPLICATES REMOVED)
L5 35 S ACYL CARRIER PROTEIN AND CHEMICAL MODIFICATION
L6 19 DUP REM L5 (16 DUPLICATES REMOVED)

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L7            19 FOCUS L6 1-
L8             0 S ACYL CARRIER PROTEIN AND APO-ACYL CARRIER PROTEIN AND HOLO-AC
L9            79 S (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACY
L10           41 DUP REM L9 (38 DUPLICATES REMOVED)

=> focus l10
PROCESSING COMPLETED FOR L10
L11           41 FOCUS L10 1-

=> d l11 1-10 ibib ab

L11  ANSWER 1 OF 41  CAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:855956  CAPLUS
DOCUMENT NUMBER:          139:347484
TITLE:                    Cloning, sequence, crystal structure and physical
                          characterization of (3R)-hydroxymyristoyl-(
                          acyl-carrier-protein)
                          dehydratase from Pseudomonas aeruginosa and its use as
                          antimicrobial target
INVENTOR(S):              Edwards, Aled; Dharamsi, Akil; Vedadi, Masoud;
                          Domagala, Megan; McDonald, Merry-Lynn; Houston, Simon;
                          Vallee, Francois; Kimber, Matthew; Awrey, Donald;
                          Beattie, Bryan
PATENT ASSIGNEE(S):       Affinium Pharmaceuticals, Inc., Can.
SOURCE:                   PCT Int. Appl., 304 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     ---------------   ----  --------      ---------------  --------
     WO 2003089463      A1   20031030      WO 2003-CA560    20030417
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
             LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,
             PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT,
             TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ,
             MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
             CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC,
             NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
             GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:                    US 2002-373321P  P  20020417
AB   The present invention relates to novel drug targets for pathogenic
     bacteria. Reliable, high throughput methods are developed to identify,
     express, and purify (3R)-hydroxymyristoyl-(acyl-carrier
     -protein) dehydratase from P. aeruginosa. The invention
     provides the nucleic acid sequence and the encoded amino acid sequence of
     the enzyme. The invention also provides crystal structure and other
     biochem. and biophys. characteristics of the P. aeruginosa
     (3R)-hydroxymyristoyl-(acyl-carrier-protein)
     dehydratase.
REFERENCE COUNT:          4     THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11  ANSWER 2 OF 41  CAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         1969:428032  CAPLUS
DOCUMENT NUMBER:          71:28032
TITLE:                    Relations between coenzyme A and presumptive
                          acyl carrier protein in
                          different conditions of streptococcal growth
AUTHOR(S):                Das, D. N.; Toennies, G.
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CORPORATE SOURCE: Sch. of Med., Temple Univ., Philadelphia, PA, USA
SOURCE: Journal of Bacteriology (1969), 98(3), 898-902
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Exploration of the specific role of cystine in the postexponential growth of Streptococcus faecalis led to an inquiry into the fate of cellular CoA and **acyl carrier protein** (ACP), both of which depend for their biosynthesis on cystine and pantothenate as precursors. In S. faecalis cells labeled by growth in the presence of 14C-labeled pantothenate, the **label** could be sepd. on the basis of soly. at pH 2.1 into 2 fractions of sharply differing metabolic characteristics. The fractions were not purified, but the sol. 14C behaved anal. like CoA, and the insol. 14C was considered to represent an ACP-like entity on the basis of circumstantial evidence. The fate of these 2 fractions under various conditions of growth was studied. When the medium contained an excess of the needed precursors, the cellular content of CoA and ACP appeared to remain const. during exponential growth, and in a molar ratio of about 4 CoA to 1 ACP. Cellular ACP, once formed, appeared to be stable under these conditions, but CoA was degraded and replaced at the rate of .apprx.20% per division period. With restrictive levels of pantothenate in the medium, initially formed CoA disappeared during growth, as a result, apparently of being converted to ACP. However, when the resulting CoA-depleted cells were returned to a medium contg. enough pantothenate, resumption of normal growth was preceded by a lag period, during which rapid conversion of ACP to CoA appeared to take place.

L11 ANSWER 3 OF 41 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:294931 CAPLUS
DOCUMENT NUMBER: 129:14445
TITLE: Expression of lauroyl-**acyl carrier protein** thioesterase in Brassica napus seeds induces pathways for both fatty acid oxidation and biosynthesis and implies a set point for triacylglycerol accumulation
AUTHOR(S): Eccleston, Victoria S.; Ohlrogge, John B.
CORPORATE SOURCE: Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI, 48824, USA
SOURCE: Plant Cell (1998), 10(4), 613-621
CODEN: PLCEEW; ISSN: 1040-4651
PUBLISHER: American Society of Plant Physiologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Expression of a California bay lauroyl-**acyl carrier protein** thioesterase (MCTE) in developing seeds of transgenic oil-seed rape alters the fatty acid compn. of the mature seed, resulting in up to 60 mol% of laurate in triacylglycerols. In this study, the authors examd. the metab. of lauric acid and 14C-acetate in developing seeds of oilseed rape that express high levels of MCTE. Lauroyl-CoA oxidase activity but not palmitoyl-CoA oxidase activity was increased severalfold in developing seeds expressing MCTE. In addn., isocitrate lyase and malate synthase activities were six- and 30-fold higher, resp., in high-laurate developing seeds. Control seeds incorporated 14C-acetate almost entirely into fatty acids, whereas in seeds expressing MCTE, only 50% of the **label** was recovered in lipids and the remainder was in a range of water-sol. components, including sucrose and malate. These results indicate that the pathways for .beta.-oxidn. and the glyoxylate cycle have been induced in seeds expressing high levels of MCTE. Although a substantial portion of the fatty acid produced in these seeds is recycled to acetyl-CoA and sucrose through the .beta.-oxidn. and glyoxylate cycle pathways, total seed oil is not reduced. How is oil content maintained if lauric acid is inefficiently converted to triacylglycerol. The levels of **acyl carrier protein** and several enzymes of fatty acid synthesis were increased

two- to threefold at midstage development in high-laurate seeds. These results indicate that a coordinate induction of the fatty acid synthesis pathway occurs, presumably to compensate for the lauric acid lost through .beta.-oxidn. or for a shortage of long-chain fatty acids.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 41 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1975:474307 CAPLUS
DOCUMENT NUMBER: 83:74307
TITLE: Separation of pigeon liver apo- and holo-fatty acid synthetases by affinity chromatography
AUTHOR(S): Qureshi, Asaf A.; Kim, Manok; Lornitzo, Frank A.; Jenik, Robert A.; Porter, John W.
CORPORATE SOURCE: Lipid Metab. Lab., VA Hosp., Madison, WI, USA
SOURCE: Biochemical and Biophysical Research Communications (1975), 64(3), 836-44
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Apo- and holo-fatty acid synthetases of pigeon liver were sepd. by affinity gel chromatog. under conditions similar to, but not identical to, those used in separating subunits I and II of pantetheine-14C-labeled fatty acid synthetase cocomplex (F.A. Lornitzo et al., 1974). When pantetheine-labeled fatty acid synthetases were sepd., the enzymically active holo form contained all of the 14C **label**. Incubation of the apo-pigeon liver fatty acid synthetase complex with CoA, ATP, and a partially purified pigeon liver sol. enzyme system, from which fatty acid synthetase was removed, resulted in the formation of holoenzyme. Activation of apo-fatty acid synthetase could also be achieved by replacing the apo-(4'-phosphopantetheine-less) **acyl carrier protein** with **holo-acyl carrier protein**. Thus, the inactive apo-fatty acid synthetase lacks a 4'-phosphopantetheine group.

L11 ANSWER 5 OF 41 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1969:111764 CAPLUS
DOCUMENT NUMBER: 70:111764
TITLE: Isolation of an **acyl carrier protein** component from the multienzyme complex of yeast fatty acid synthetase
AUTHOR(S): Willecke, Klaus; Ritter, E.; Lynen, Feodor
CORPORATE SOURCE: Max-Planck-Inst. Zellchem., Munich, Fed. Rep. Ger.
SOURCE: European Journal of Biochemistry (1969), 8(4), 503-9
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Pantetheine-14C-fatty acid synthetase from bakers' yeast was denatured in guanidine-HCl. Then the protein components of the multienzyme complex were subjected to a Sephadex filtration in guanidine-HCl and a preparative polyacrylamide gel electrophoresis in a phenol-contg. medium. It could be demonstrated that the pantetheine-14C **label** was covalently bound to 1 of the smallest polypeptide chains of the multienzyme complex. The pantetheine-protein component of the yeast multienzyme complex was denoted as yeast **acyl carrier protein**. Its mol. weight seemed to be higher and its amino acid compn. different from the Escherichia coli **acyl carrier protein**.

L11 ANSWER 6 OF 41 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1984:350604 BIOSIS
DOCUMENT NUMBER: PREV198478087084; BA78:87084
TITLE: IN-VITRO FATTY-ACID SYNTHESIS AND COMPLEX LIPID METABOLISM IN THE CYANOBACTERIUM ANABAENA-VARIABILIS 2. ACYL TRANSFER AND COMPLEX LIPID FORMATION.
AUTHOR(S): LEM N W [Reprint author]; STUMPF P K

CORPORATE SOURCE: DEP BIOCHEM AND BIOPHYSICS, UNIV CALIF, DAVIS, CALIF 95616, USA
SOURCE: Plant Physiology (Rockville), (1984) Vol. 75, No. 3, pp. 700-704.
CODEN: PLPHAY. ISSN: 0032-0889.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB In vitro fatty acid transfer to form complex lipids was observed in crude cell extracts of A. variabilis using [1-14C]palmitoyl-**acyl carrier protein**, [1-14C]stearoyl-**acyl carrier protein** and [1-14C]oleoyl-**acyl carrier protein** substrates. There apparently was a rapid transfer of the fatty acids into the complex lipids. The greatest amount of radioactivity was observed in the monogalactosyl diacylglycerol fractions and there appeared to be a preference for the transfer of stearate over palmitate. The exogenously added lysophospholipids (lysophosphatidylglycerol, lysophosphatidylcholine) and 2-monopalmitin acted as acceptors in acyl transfer. Addition of the hypolipidemic drug, WY14643, inhibited the fast acyl transfer reaction and showed that the 1st product of acyl transfer was diglyceride followed by monogalactosyl diacylglycerol. Thioesters of coenzyme A do not seem to be involved in these reactions.

L11 ANSWER 7 OF 41 MEDLINE on STN
ACCESSION NUMBER: 83023267 MEDLINE
DOCUMENT NUMBER: 83023267 PubMed ID: 6922724
TITLE: Isolation and characterization of an **acyl carrier protein** from pigeon liver fatty acid synthetase by controlled proteolysis with elastase.
AUTHOR: Puri R N; Porter J W
CONTRACT NUMBER: AM 01383 (NIADDK)
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1982 Sep 14) 712 (3) 576-89.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198212
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 20000303
Entered Medline: 19821218

AB Controlled proteolytic cleavage of 4'-phospho[14C]pantetheine-labeled pigeon liver fatty acid synthetase generates two 4'-phospho[14C]pantetheine-labeled peptides, Ec1 and Ec2. These are separated from each other and the core enzyme by gel permeation chromatography on a Sephadex G-75 column. The two radioactively labeled peptides constitute 50% of the radioactivity initially present in the 4'-phospho[14C]pantetheine-labeled fatty acid synthetase. The remaining **label** in the core enzyme is released quantitatively by proteolytic cleavage with trypsin. The molecular weights of Ec1 and Ec2 peptides, as determined by size exclusion chromatography and SDS-polyacrylamide gel electrophoresis, are 12000 and 6000, respectively. Both the higher and lower molecular weight peptides are homogeneous with respect to size and charge, as shown by polyacrylamide gel electrophoresis in the presence and absence of SDS. The higher molecular weight peptide, Ec1, is characterized as an **acyl carrier protein** by the transacylation reaction between the unlabeled Ec1 peptide and radioactively labeled acetyl- and malonyl-CoA. Since Ec2 peptide also contains the prosthetic group present in the Ec1 peptide, the Ec2 peptide appears to result from the proteolytic cleavage of the higher molecular weight peptide, Ec1. Amino acid composition of the **acyl carrier protein** shows the presence of 1 mol of 4'-phosphopantetheine per mol of protein. 2 mol of **acyl**

carrier protein are present per mol of the fatty acid synthetase. The amino acid analysis is in good agreement with the molecular weight of the Ec1 peptide, as determined by gel filtration and SDS-polyacrylamide gel electrophoresis. N-Terminal amino acid analysis of this peptide shows the presence of an arginine residue.

L11 ANSWER 8 OF 41 MEDLINE on STN
ACCESSION NUMBER: 2001073203 MEDLINE
DOCUMENT NUMBER: 20504166 PubMed ID: 11049751
TITLE: Chemical and posttranslational modification of Escherichia coli **acyl carrier protein** for preparation of dansyl-acyl carrier proteins.
AUTHOR: Haas J A; Frederick M A; Fox B G
CORPORATE SOURCE: Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, USA.
CONTRACT NUMBER: GM50853 (NIGMS)
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (2000 Nov) 20 (2) 274-84.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010103

AB Escherichia coli **acyl carrier protein** (ACP) contains a single tyrosine residue at position 71. The combined o-nitration of apo-ACP Y71 by tetranitromethane and reduction to 3-aminotyrosyl-apo-ACP were performed to introduce a specific site for attachment of a dansyl fluorescent **label**. Conditions for purification and characterization of dansylaminotyrosyl-apo-ACP are reported. Dansylaminotyrosyl-apo-ACP was enzymatically phosphopantetheinylated and acylated in vitro with an overall approximately 30% yield of purified stearoyl-dansylaminotyrosyl-ACP starting from unmodified apo-ACP. The steady-state kinetic parameters k(cat) = 22 min(-1) and K(M) = 2.7 microM were determined for reaction of stearoyl-dansylaminotyrosyl-ACP with stearoyl-ACP Delta(9)-desaturase. These results show that dansylaminotyrosyl-ACP will function well for studying binding interactions with the Delta(9)-desaturase and suggest similar possibilities for other ACP-dependent enzymes. The efficient in vivo phosphopantetheinylation of E. coli apo-ACP by coexpression with holo-ACP synthase in E. coli BL21(DE3) using fructose as the carbon source is also reported.

L11 ANSWER 9 OF 41 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1972:416427 CAPLUS
DOCUMENT NUMBER: 77:16427
TITLE: Biosynthesis of unsaturated fatty acids by bacilli. III. Uptake and utilization of exogenous palmitate
AUTHOR(S): Fulco, Armand J.
CORPORATE SOURCE: Lab. Nucl. Med. Radiat. Biol., Univ. California, Los Angeles, CA, USA
SOURCE: Journal of Biological Chemistry (1972), 247(11), 3503-10
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The factors affecting the uptake, utilization, and desatn. of exogenous palmitic acid by Bacillus megaterium 14581 have been elucidated and the validity of the in vivo assay for detg. the relative levels of the temp.-induced 5-desatg. enzyme system in this organism has been established. It was found that, in the presence of glucose,

[1-14C]-palmitic acid added to the cultures of B. megaterium is taken up rapidly and quant. by the cells. However, most of the 14C **label** is excreted back into the medium within 15 min as water-sol. products (CO2, acetate, etc.) presumably derived from .beta. oxidn. of the added [1-14C]-palmitate. The remaining [1-14C]-palmitate (about 30% of the amt. added) mixes with a small endogenous pool of palmitate (approx. 35 m.mu. moles per g of cells) which is readily desatd. but is completely stable to .beta. oxidn. In the absence of glucose, .beta. oxidn. of added [1-14C]-palmitate follows the same time pattern but a greater proportion of the **label** is excreted into the medium as oxidn. products. Concomitantly, the endogenous pool of palmitate available for desatn. drops to 0 and total desatn. is reduced to about 6 to 7% of that obtained in the presence of glucose. Considerable exptl. evidence would indicate that neither palmityl-CoA nor the palmitate of neutral lipids or phospholipids is a substrate for desatn. It seems more probable that the true substrate for desatn. is the palmitate derived from fatty acid biosynthesis, presumably the palmityl thioester of **acyl carrier protein**. Exogenously derived [1-14C]-palmitate is thought to enter this pool indirectly by first being incorporated into a small, metabolically active pool of neutral lipids and phospholipids and then exchanging rapidly with endogenous palmityl **acyl carrier protein**. In the presence of glucose, the endogenous pool of palmitate available for desatn. was shown to remain const. during the period when 5-desatg. enzyme was induced from an undetectable level to max. levels after transfer of a 35.degree.-grown culture of B. megaterium to 20.degree.. Furthermore, the pool size was shown to be independent of levels of substrate desatn. In all cases, in the presence of glucose and chloramphenicol, the level of desatn. was detd. solely by the level of 5-desatg. enzyme within wide exptl. limits. This fact permitted the development of a precise and accurate assay procedure for the detn. of relative desatg. enzyme levels and opened the way for the in vivo study of the temp.-mediated mechanisms which regulate unsatd. fatty acid biosynthesis in B. megaterium.

L11 ANSWER 10 OF 41 MEDLINE on STN

ACCESSION NUMBER:	2002437930 MEDLINE
DOCUMENT NUMBER:	22163960 PubMed ID: 12173933
TITLE:	Enzymes involved in fatty acid and polyketide biosynthesis in Streptomyces glaucescens: role of FabH and FabD and their **acyl carrier protein** specificity.
AUTHOR:	Florova Galina; Kazanina Galina; Reynolds Kevin A
CORPORATE SOURCE:	Department for Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, Virginia 23219, USA.
CONTRACT NUMBER:	GM50541 (NIGMS)
SOURCE:	BIOCHEMISTRY, (2002 Aug 20) 41 (33) 10462-71. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY:	United States
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	200209
ENTRY DATE:	Entered STN: 20020829 Last Updated on STN: 20020906 Entered Medline: 20020905

AB Malonyl **acyl carrier protein** (ACP) is used as an extender unit in each of the elongation steps catalyzed by the type II dissociated fatty acid synthase (FAS) and polyketide synthase (PKS) of Streptomyces glaucescens. Initiation of straight-chain fatty acid biosynthesis by the type II FAS involves a direct condensation of acetyl-CoA with this malonyl-ACP to generate a 3-ketobutyryl-ACP product and is catalyzed by FabH. In vitro experiments with a reconstituted type II PKS system in the absence of FabH have previously shown that the acetyl-ACP (generated by decarboxylation of malonyl-ACP), not acetyl-CoA,

is used to initiate tetracenomycin C (TCM C) biosynthesis. We have shown that sgFabH activity is present in S. glaucescens fermentations during TCM C production, suggesting that it could contribute to initiation of TCM C biosynthesis in vivo. Isotope incorporation studies with [CD3]acetate and [13CD3]acetate demonstrated significant intact retention of three deuteriums into the starter unit of palmitate and complete washout of deuterium **label** into the starter unit of TCM C. These observations provide evidence that acetyl-CoA is not used directly as a starter unit for TCM C biosynthesis in vivo and argue against an involvement of FabH in this process. Consistent with this conclusion, assays of the purified recombinant sgFabH with acetyl-CoA demonstrated activity using malonyl-ACP generated from either FabC (the S. glaucescens FAS ACP) (k(cat) 42.2 min(-1), K(m) 4.5 +/- 0.3 microM) or AcpP (the E. coli FAS ACP) (k(cat) 7.5 min(-1), K(m) 6.3 +/- 0.3 microM) but not TcmM (the S. glaucescens PKS ACP). In contrast, the sgFabD which catalyzes conversion of malonyl-CoA to malonyl-ACP for fatty acid biosynthesis was shown to be active with TcmM (k(cat) 150 min(-1), K(m) 12.2 +/- 1.2 microM), AcpP (k(cat) 141 min(-1), K(m) 13.2 +/- 1.6 microM), and FabC (k(cat) 560 min(-1), K(m) 12.7 +/- 2.6 microM). This enzyme was shown to be present during TCM C production and could play a role in generating malonyl-ACP for both processes. Previous demonstrations that the purified PKS ACPs catalyze self-malonylation and that a FabD activity is not required for polyketide biosynthesis are shown to be an artifact of the expression and purification protocols. The relaxed ACP specificity of FabD and the lack of a clear alternative are consistent with a role of FabD in providing malonyl-ACP precursors for PKS as well as FAS processes. In contrast, the ACP specificity of FabH, isotope labeling studies, and a demonstrated alternative mechanism for initiation of the PKS process provide unequivocal evidence that FabH is involved only in the FAS process.

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=> s (acyl carrier protein or apo-acyl carrier protein or holo-acyl carrier protein or a
L12          37 (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACYL
               CARRIER PROTEIN OR ACYLATED ACYL CARRIER PROTEIN) AND FLUORESCEN
               T

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13          23 DUP REM L12 (14 DUPLICATES REMOVED)

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PROCESSING COMPLETED FOR L13
L14          23 FOCUS L13 1-

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L14  ANSWER 1 OF 23  CAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2003:498728  CAPLUS
DOCUMENT NUMBER:          140:14221
TITLE:                    A continuous coupled enzyme assay for bacterial
                          malonyl-CoA:acyl carrier
                          protein transacylase (FabD)
AUTHOR(S):                Molnos, Juliette; Gardiner, Rana; Dale, Glenn E.;
                          Lange, Roland
CORPORATE SOURCE:         Morphochem AG Basel, Basel, CH-4058, Switz.
SOURCE:                   Analytical Biochemistry (2003), 319(1), 171-176
                          CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER:                Elsevier Science
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   Bacterial malonyl-CoA:acyl carrier protein
     transacylase catalyzes the transfer of a malonyl moiety from malonyl-CoA
     to the free thiol group of the phosphopantetheine arm of acyl
     carrier protein. Malonyl-ACP, the product of this
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enzymic reaction, is the key building block for de novo fatty acid biosynthesis. Here, we describe a continuous enzyme assay based on the coupling of the malonyl-CoA:**acyl carrier protein** transacylase reaction to .alpha.-ketoglutarate dehydrogenase (KDH). KDH-dependent consumption of the CoA generated by malonyl-CoA:**acyl carrier protein** transacylase is accompanied by a redn. of NAD, oxidized (NAD+) to NAD, reduced. The rate of NAD+ redn. is continuously monitored as a change in fluorescence using a microtiter plate reader. We show that this coupled enzyme assay is amenable to routine chem. compd. screening.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 2 OF 23 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-12861 BIOTECHDS
TITLE: New soluble triacylglycerol (TAG) biosynthetic enzymes in eukaryotic cells, useful for identifying compounds that are capable of altering TAG synthesis and accumulation, or treating an imbalance in TAG levels, e.g. atherosclerosis; drug screening and potential gene therapy of atherosclerosis and hyperlipidemia
AUTHOR: RAJASHEKARAN R
PATENT ASSIGNEE: BIJAM BIOSCIENCES LTD; INDIAN INST SCI
PATENT INFO: WO 2003018814 6 Mar 2003
APPLICATION INFO: WO 2002-IN176 29 Aug 2002
PRIORITY INFO: US 2001-315757 30 Aug 2001; US 2001-315757 30 Aug 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-290074 [28]

AB DERWENT ABSTRACT:

NOVELTY - Soluble triacylglycerol biosynthetic enzymes in eukaryotic cells comprising of lysophosphatidic acid acyltransferase, phosphatidic acid phosphatase, diacylglycerol acyltransferase, acyl-**acyl carrier protein** synthetase, superoxide dismutase, and **acyl carrier protein**, are new.

DETAILED DESCRIPTION - A lysophosphatidic acid acyltransferase comprising the peptide sequences (S1) and (S3): (i) X-Ala-Leu-Glu-Leu-Gln-Ala-Asp-Asp-Phe-Asn-Lys (S1); and (ii) X-X-Val-Asn-Asn-Val-X-Pro-Gly-X-Ile-Glu Gln (S3). A phosphatidic acid phosphatase comprising the peptide sequences (S4) and (S5): (i) Asn-Ala-Leu-Thr-Gly-Leu-His-Met-Gly-Gly-Gly-Lys (S4); and (ii) Tyr-Val-Glu Gly-Ala-Arg Pro-X-Lys (S5). A diacylglycerol acyltransferase comprising the peptide sequence (S7) and is encoded by a fully defined sequence of 1238 bp (S11) given in the specification: X-Leu-Trp-Ala-Val-Val-Gly-Ala-Gln-Pro-Phe-Gly-Gly-Ala-Arg-Gly-Ser (S7) A acyl-**acyl carrier protein** synthetase comprising the peptide sequence (S8): Val-His-Leu-Ala-Val-Ala Leu-Tyr-Gly-Leu-Ala-Ala-Val-Arg-Val-Ser-Arg-Ile-Val-Arg (S8) The superoxide dismutase is encoded by a fully defined sequence of 733 bp (S9) given in the specification. The **acyl carrier protein** is encoded by a fully defined sequence of 565 bp (S10) given in the specification. INDEPENDENT CLAIMS are included for: (1) polypeptides of: (a) superoxide dismutase (SOD) that is encoded by the gene sequence of S9; (b) **acyl carrier protein** (ACP) that is encoded by the gene sequence of S10; or (c) diacylglycerol acyltransferase that is encoded by the gene sequence of S11; (2) an oligopeptide of at least 5-20 amino acids of the peptides S1-S8 capable of specifically identifying with a unique sequence of proteins present within a triacylglycerol biosynthetic complex (TBC); (3) antibodies to at least 5-20 amino acids of the peptides S1-S8 from the isolated polypeptides from TBC, which are capable of specifically identifying the proteins present within a TBC; (4) an oligonucleotide of at least 15 nucleotides from the sequence of S9 or S10, capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid, which encodes SOD or ACP, respectively; (5) a nucleic acid having a sequence complementary to the sequences of S9-S11, which encode

SOD, ACP and diacylglycerol acyltransferase; (6) an in vitro method of detecting the soluble triacylglycerol (TAG) biosynthetic enzymes, which exist as free or as a complex in the cytosol; (7) a method for determining whether a subject known to have an imbalance in TAG has the imbalance due to a defect in the synthesis of TAG; (8) a method for treating a subject who has an imbalance in triglyceride or TAG levels due to a defect in the synthesis of soluble triglyceride, comprising introducing the isolated nucleic acid that encodes lysophosphatidic acid acyltransferase, phosphatidic acid phosphatase, diacylglycerol acyltransferase, ACP, acyl-ACP synthetase into the subject under conditions such that the nucleic acid expresses the soluble TAG biosynthetic enzymes individually or in combination; (9) a method for inhibiting the soluble TAG biosynthetic enzymes in a subject comprising: (a) transforming appropriate cells from the subject with a vector that expresses the nucleic acid, which encodes the components in TBC, and introducing the transformed cells into the subject to inhibit the soluble enzyme and/or complex; or (b) introducing any of the oligonucleotides cited above of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid encoding lysophosphatidic acid acyltransferase, phosphatidic acid phosphatase, diacylglycerol acyltransferase, ACP, acyl-ACP synthetase and SOD into the subject to inhibit the triglyceride formation and accumulation; (10) a method of obtaining soluble TAG biosynthetic enzymes using polyacrylamide gel electrophoresis, chromatographic procedures or density gradient centrifugation comprising subjecting blue sepharose, DEAE matrix octail sepharose, antibodies raised from the peptides S1-S8, and identifying and isolating the complex as a whole or individual components.

BIOTECHNOLOGY - Preferred Enzyme: The soluble triacylglycerol (TAG) biosynthetic enzymes are soluble in aqueous solutions. The soluble TAG enzymes in eukaryotic cells, where the eukaryotic cells are preferably oleaginous yeast, backer's yeast, rat adipocytes and human cell-lines (HepG2). The soluble TAG enzymes in oleaginous yeast comprises lysophosphatidic acid acyltransferase, phosphatidic acid phosphatase, diacylglycerol acyltransferase, acyl-**acyl carrier protein** synthetase, superoxide dismutase, and **acyl carrier protein**, where the gene sequence encoding superoxide dismutase, **acyl carrier protein** and diacylglycerol acyltransferase have sequence homology to DNA sequence of S9, S10 and S11, respectively. Lysophosphatidic acid acyltransferase, diacylglycerol acyltransferase, and phosphatidic acid phosphatase are identified by immunological cross reactivity to the peptide sequence (S2), S7, and S4 and S6, respectively: Ala-Leu-Glu Leu-Gln-Ala Asp-Asp-Phe Asn-Lys (S2) The enzymes are responsible for TAG synthesis and accumulation in free or multi enzyme complex form. Preferred Polypeptide: The polypeptides of superoxide dismutase, **acyl carrier protein** and diacylglycerol acyltransferase are prepared by expressing the gene of S9, S10 and S11, respectively, in Escherichia coli.

ACTIVITY - Antilipemic; Antiarteriosclerotic. No biological data given.

MECHANISM OF ACTION - Enzyme.

USE - The soluble triacylglycerol (TAG) biosynthetic enzymes are useful for identifying compounds that are capable of altering TAG synthesis and accumulation, or for treating a subject who has an imbalance in triglyceride or TAG levels, e.g. atherosclerosis or hyperlipidemia. The TAG biosynthetic enzyme sequences are useful as target sites for controlling fat (all claimed).

EXAMPLE - The growth of oleaginous yeast cells were monitored, and yeast cells grown at various time intervals were stained with **fluorescent** dye (Nile blue A) followed by phase contrast fluorescence microscopy of cells revealing that 24-hour grown cells accumulate triacylglycerol (TAG) to the lesser extent. Stationary phase cells showed an intense Nile blue A staining suggesting the large accumulation of TAG at the stationary phase. Lipids were extracted and

separated by silica-TLC using neutral lipid solvent system and the results indicated that TAG accumulation was found even at the early logarithmic phase. Of the various TAG biosynthetic enzymes assayed, the soluble fraction exhibited high amounts (49-69% total activity) of lysophosphatidic acid acyltransferase, diacylglycerol acyltransferase and phosphatidic acid phosphatase activities as compared to the corresponding enzymes in the particulate fraction. These results indicated that an additional TAG biosynthetic pathway could exist in the soluble fraction.(59 pages)

L14 ANSWER 3 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2003466211 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12882974
TITLE: Cloning, expression, characterization, and interaction of two components of a human mitochondrial fatty acid synthase. Malonyltransferase and **acyl carrier protein**.
AUTHOR: Zhang Lei; Joshi Anil K; Smith Stuart
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, Oakland, California 94609.
CONTRACT NUMBER: DK 16073 (NIDDK)
SOURCE: Journal of biological chemistry, (2003 Oct 10) 278 (41) 40067-74.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200312
ENTRY DATE: Entered STN: 20031008
Last Updated on STN: 20031218
Entered Medline: 20031202

AB The possibility that human cells contain, in addition to the cytosolic type I fatty acid synthase complex, a mitochondrial type II malonyl-CoA-dependent system for the biosynthesis of fatty acids has been examined by cloning, expressing, and characterizing two putative components. Candidate coding sequences for a malonyl-CoA:**acyl carrier protein** transacylase (malonyltransferase) and its **acyl carrier protein** substrate, identified by BLAST searches of the human sequence data base, were located on nuclear chromosomes 22 and 16, respectively. The encoded proteins localized exclusively in mitochondria only when the putative N-terminal mitochondrial targeting sequences were present as revealed by confocal microscopy of HeLa cells infected with appropriate green **fluorescent** protein fusion constructs. The mature, processed forms of the mitochondrial proteins were expressed in Sf9 cells and purified, the **acyl carrier protein** was converted to the holoform in vitro using purified human phosphopantetheinyltransferase, and the functional interaction of the two proteins was studied. Compared with the dual specificity malonyl/acetyltransferase component of the cytosolic type I fatty acid synthase, the type II mitochondrial counterpart exhibits a relatively narrow substrate specificity for both the acyl donor and **acyl carrier protein** acceptor. Thus, it forms a covalent acyl-enzyme complex only when incubated with malonyl-CoA and transfers exclusively malonyl moieties to the mitochondrial holoacyl carrier protein. The type II **acyl carrier protein** from Bacillus subtilis, but not the **acyl carrier protein** derived from the human cytosolic type I fatty acid synthase, can also function as an acceptor for the mitochondrial transferase. These data provide compelling evidence that human mitochondria contain a malonyl-CoA/**acyl carrier protein**-dependent fatty acid synthase system, distinct from the type I cytosolic fatty acid synthase, that resembles the type II system present in prokaryotes and plastids. The final products of this system,

yet to be identified, may play an important role in mitochondrial function.

L14 ANSWER 4 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2001169901 MEDLINE
DOCUMENT NUMBER: 21167841 PubMed ID: 11139581
TITLE: Isolation and localization of a cytosolic 10 S triacylglycerol biosynthetic multienzyme complex from oleaginous yeast.
AUTHOR: Gangar A; Karande A A; Rajasekharan R
CORPORATE SOURCE: Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Mar 30) 276 (13) 10290-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20030105
Entered Medline: 20010510

AB Triacylglycerol is one of the major storage forms of metabolic energy in eukaryotic cells. Biosynthesis of triacylglycerol is known to occur in membranes. We report here the isolation, purification, and characterization of a catalytically active cytosolic 10 S multienzyme complex for triacylglycerol biosynthesis from Rhodotorula glutinis during exponential growth. The complex was characterized and was found to contain lysophosphatidic acid acyltransferase, phosphatidic acid phosphatase, diacylglycerol acyltransferase, acyl-**acyl carrier protein** synthetase, and **acyl carrier protein**. The 10 S triacylglycerol biosynthetic complex rapidly incorporates free fatty acids as well as fatty acyl-coenzyme A into triacylglycerol and its biosynthetic intermediates. Lysophosphatidic acid acyltransferase, phosphatidic acid phosphatase, and diacylglycerol acyltransferase from the complex were microsequenced. Antibodies were raised against the synthetic peptides corresponding to lysophosphatidic acid acyltransferase and phosphatidic acid phosphatase sequences. Immunoprecipitation and immunolocalization studies show the presence of a cytosolic multienzyme complex for triacylglycerol biosynthesis. Chemical cross-linking studies revealed that the 10 S multienzyme complex was held together by protein-protein interactions. These results demonstrate that the cytosol is one of the sites for triacylglycerol biosynthesis in oleaginous yeast.

L14 ANSWER 5 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2001073203 MEDLINE
DOCUMENT NUMBER: 20504166 PubMed ID: 11049751
TITLE: Chemical and posttranslational modification of Escherichia coli **acyl carrier protein** for preparation of dansyl-acyl carrier proteins.
AUTHOR: Haas J A; Frederick M A; Fox B G
CORPORATE SOURCE: Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, USA.
CONTRACT NUMBER: GM50853 (NIGMS)
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (2000 Nov) 20 (2) 274-84.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322

Last Updated on STN: 20010322
Entered Medline: 20010103

AB Escherichia coli **acyl carrier protein** (ACP) contains a single tyrosine residue at position 71. The combined o-nitration of apo-ACP Y71 by tetranitromethane and reduction to 3-aminotyrosyl-apo-ACP were performed to introduce a specific site for attachment of a dansyl **fluorescent** label. Conditions for purification and characterization of dansylaminotyrosyl-apo-ACP are reported. Dansylaminotyrosyl-apo-ACP was enzymatically phosphopantetheinylated and acylated in vitro with an overall approximately 30% yield of purified stearoyl-dansylaminotyrosyl-ACP starting from unmodified apo-ACP. The steady-state kinetic parameters k(cat) = 22 min(-1) and K(M) = 2.7 microM were determined for reaction of stearoyl-dansylaminotyrosyl-ACP with stearoyl-ACP Delta(9)-desaturase. These results show that dansylaminotyrosyl-ACP will function well for studying binding interactions with the Delta(9)-desaturase and suggest similar possibilities for other ACP-dependent enzymes. The efficient in vivo phosphopantetheinylation of E. coli apo-ACP by coexpression with holo-ACP synthase in E. coli BL21(DE3) using fructose as the carbon source is also reported.

=> d l14 6-23 ibib ab

L14 ANSWER 6 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2003009419 MEDLINE
DOCUMENT NUMBER: 22403682 PubMed ID: 12515551
TITLE: Amino acid residues of Escherichia coli **acyl carrier protein** involved in heterologous protein interactions.
AUTHOR: Worsham Lesa M S; Earls Laurie; Jolly Carrie; Langston Keisha Gordon; Trent M Stephen; Ernst-Fonberg M Lou
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee 37614, U SA.
CONTRACT NUMBER: R01-GM62121 (NIGMS)
R15-GMOD54337 (NIGMS)
SOURCE: BIOCHEMISTRY, (2003 Jan 14) 42 (1) 167-76.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20030108
Last Updated on STN: 20030302
Entered Medline: 20030228

AB **Acyl carrier protein** (ACP) is a small, highly conserved protein with an essential role in a myriad of reactions throughout lipid metabolism in plants and bacteria where it interacts with a remarkable diversity of proteins. The nature of the proper recognition and precise alignment between the protein moieties of ACP and its many interactive proteins is not understood. Residues conserved among ACPs from numerous plants and bacteria were considered as possibly being crucial to ACP's function, including protein-protein interaction, and a method of identifying amino acid residue clusters of high hydrophobicity on ACP's surface was used to estimate residues possibly involved in specific ACP-protein interactions. On the basis of this information, single-site mutation analysis of multiple residues, one at a time, of ACP was used to probe the identities of potential contact residues of ACPSH or acyl-ACP involved in specific interactions with selected enzymes. The roles of particular ACP residues were more precisely defined by site-directed fluorescence analyses of various myristoyl-mutant-ACPs upon specific interaction with the Escherichia coli hemolysin-activating

acyltransferase, HlyC. This was done by selectively labeling each mutated site, one at a time, with an environmentally sensitive fluoroprobe and observing its fluorescence behavior in the absence and presence of HlyC. Consequently, a picture of the portion of ACP involved in selected macromolecular interaction has emerged.

L14 ANSWER 7 OF 23 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1998:715267 SCISEARCH
THE GENUINE ARTICLE: 119JT
TITLE: A new metabolic link between fatty acid de novo synthesis and polyhydroxyalkanoic acid synthesis - The phaG gene from Pseudomonas putida KT2440 encodes a 3-hydroxyacyl-**acyl carrier protein** coenzyme
A transferase
AUTHOR: Rehm B H A; Kruger N; Steinbuchel A (Reprint)
CORPORATE SOURCE: UNIV MUNSTER, INST MIKROBIOL, CORRENSSTR 3, D-48149 MUNSTER, GERMANY (Reprint); UNIV MUNSTER, INST MIKROBIOL, D-48149 MUNSTER, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (11 SEP 1998) Vol. 273, No. 37, pp. 24044-24051.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To investigate the metabolic link between fatty acid de novo synthesis and polyhydroxyalkanoic acid (PHA) synthesis, we isolated mutants of Pseudomonas putida KT2440 deficient in this metabolic route. The gene phaG was cloned by phenotypic complementation of these mutants; it encoded a protein of 295 amino acids with a molecular mass of 33,876 Da, and the amino acid sequence exhibited 44% amino acid identity to the primary structure of the rhlA gene product, which is involved in the rhamnolipid biosynthesis in Pseudomonas aeruginosa PG201. S-1 nuclease protection assay identified the transcriptional start site 239 base pairs upstream of the putative translational start codon, Transcriptional induction of phaG was observed when gluconate was provided, and PHA synthesis occurred from this carbon source. No complementation of the rhlA mutant P. aeruginosa UO299-harboring plasmid pBHR81, expressing phaG gene under lac promoter control, was obtained. Heterologous expression of phaG in Pseudomonas oleovorans, which is not capable of PHA synthesis from gluconate, enabled PHA synthesis on gluconate as the carbon source, Native recombinant PhaG was purified by native polyacrylamide gel electrophoresis from P. oleovorans-harborinag plasmid pBHR81. It catalyzes the transfer of the acyl moiety from in vitro synthesized 3-hydroxydecanoyl-CoA to **acyl carrier protein**, indicating that PhaG exhibits a 3-hydroxyacyl-CoA-**acyl carrier protein** transferase activity.

L14 ANSWER 8 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2002702655 MEDLINE
DOCUMENT NUMBER: 22351773 PubMed ID: 12463745
TITLE: Fluorescence anisotropy studies of enzyme-substrate complex formation in stearoyl-ACP desaturase.
AUTHOR: Haas Jeffrey A; Fox Brian G
CORPORATE SOURCE: Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706, USA.
CONTRACT NUMBER: GM-50853 (NIGMS)
SOURCE: BIOCHEMISTRY, (2002 Dec 10) 41 (49) 14472-81.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021217
Last Updated on STN: 20030125
Entered Medline: 20030124

AB Stearoyl-**acyl carrier protein** Delta(9)-desaturase (delta9D) catalyzes regio- and stereospecific insertion of cis double bonds into acyl chains attached to **acyl carrier protein**. Steady-state and stopped-flow fluorescence anisotropy measurements using acylated forms of dansyl- and fluoresceinyl-ACPs revealed equilibrium dissociation constants and dissociation rate constants for 16:0-, 17:0-, and 18:0-ACPs with resting and chemically 4e(-) reduced delta9D. Binding of 1 nM 18:0-fluoresceinyl-ACP to one subunit of the dimeric resting delta9D was observed with K(D1) = 13 +/- 3 nM. No significant difference in the K(D1) value was observed for 4e(-) delta9D. An approximately 4-fold increase in K(D1) per methylene group was observed upon shortening the acyl chain from 18:0 to 17:0 and then 16:0. In different experiments performed with 850 nM 18:0-dansyl-ACP, binding to the second subunit of resting delta9D was estimated to have K(D2) approximately 350 +/- 40 nM. The K(D2) values exhibited a similar dependence on acyl chain length as observed for the K(D1) values. The k(off) values measured by stopped-flow anisotropy measurements for reversal of the enzyme-substrate complex were also acyl-chain length dependent and increased 130-fold for 16:0-ACP (130 s(-)(1)) relative to 18:0-ACP (1 s(-)(1)). Increases in acyl chain length are thus associated with the presently reported increases in the K(D) and k(off) values. These results indicate that acyl chain length selectivity derives in major part from partition of the enzyme-substrate complex between substrate release and subsequent steps in catalysis.

L14 ANSWER 9 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2000155414 MEDLINE
DOCUMENT NUMBER: 20155414 PubMed ID: 10693747
TITLE: Molecular analysis of a Type I fatty acid synthase in Cryptosporidium parvum.
AUTHOR: Zhu G; Marchewka M J; Woods K M; Upton S J; Keithly J S
CORPORATE SOURCE: New York State Department of Health, Wadsworth Center, Albany 12201-2002, USA.. zhug@wadsworth.org
CONTRACT NUMBER: AI40320 (NIAID)
SOURCE: MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (2000 Feb 5) 105 (2) 253-60.
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF082993
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000505
Last Updated on STN: 20000505
Entered Medline: 20000424

AB We report here the molecular analysis of a Type I fatty acid synthase in the apicomplexan Cryptosporidium parvum (CpFAS1). The CpFAS1 gene encodes a multifunctional polypeptide of 8243 amino acids that contains 21 enzymatic domains. This CpFAS1 structure is distinct from that of mammalian Type I FAS, which contains only seven enzymatic domains. The CpFAS1 domains are organized into: (i) a starter unit consisting of a fatty acid ligase and an **acyl carrier protein** ; (ii) three modules, each containing a complete set of six enzymes (acyl transferase, ketoacyl synthase, ketoacyl reductase, dehydrase, enoyl reductase, and **acyl carrier protein**) for the elongation of fatty acid C2-units; and (iii) a terminating domain whose function is as yet unknown. The CpFAS1 gene is expressed throughout the

life cycle of C. parvum, since its transcripts and protein were detected by RT-PCR and immunofluorescent localization, respectively. This cytosolic Type I CpFAS1 differs from the organellar Type II FAS enzymes identified from Toxoplasma gondii and Plasmodium falciparum which are targetted to the apicoplast, and are sensitive to inhibition by thiolactomycin. That the discovery of CpFAS1 may provide a new biosynthetic pathway for drug development against cryptosporidiosis, is indicated by the efficacy of the FAS inhibitor cerulenin on the growth of C. parvum in vitro.

L14 ANSWER 10 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2003595287 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 14675432
TITLE: Organisation of the pantothenate (vitamin B5) biosynthesis pathway in higher plants.
AUTHOR: Ottenhof Harald H; Ashurst Jennifer L; Whitney Heather M; Saldanha S Adrian; Schmitzberger Florian; Gweon Hyun Soon; Blundell Tom L; Abell Chris; Smith Alison G
CORPORATE SOURCE: Department of Plant Sciences, Downing Street, Cambridge CB2 3EA, UK, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK, and Department of Biochemistry, 80 Tennis Court Road, Cambridge CB2 1GA, UK.
SOURCE: Plant journal : for cell and molecular biology, (2004 Jan) 37 (1) 61-72.
Journal code: 9207397. ISSN: 0960-7412.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20031217
Last Updated on STN: 20031217

AB Pantothenate (vitamin B5) is the precursor for the biosynthesis of the phosphopantetheine moiety of coenzyme A and **acyl carrier protein**, and is synthesised in Escherichia coli by four enzymic reactions. Ketopantoate hydroxymethyltransferase (KPHMT) and pantothenate synthetase (PtS) catalyse the first and last steps, respectively. Two genes encoding KPHMT and one for PtS were identified in the Arabidopsis thaliana genome, and cDNAs for all three genes were amplified by PCR. The cDNAs were able to complement their respective E. coli auxotrophs, demonstrating that they encoded functional enzymes. Subcellular localisation of the proteins was investigated using green **fluorescent** protein (GFP) fusions and confocal microscopy. The two KPHMT-GFP fusion proteins were targeted exclusively to mitochondria, whereas PtS-GFP was found in the cytosol. This implies that there must be transporters for pathway intermediates. KPHMT enzyme activity could be measured in purified mitochondria from both pea leaves and Arabidopsis suspension cultures. We investigated whether Arabidopsis encoded homologues of the remaining two pantothenate biosynthesis enzymes from E. coli, l-aspartate-alpha-decarboxylase (ADC) and ketopantoate reductase (KPR). No homologue of ADC could be identified using either conventional blast or searches with the program fugue in which the structure of the E. coli ADC was compared to all the annotated proteins in Arabidopsis. ADC also appears to be absent from the genome of the yeast, Saccharomyces cerevisiae, by the same criteria. In contrast, a putative Arabidopsis oxidoreductase with some similarity to KPR was identified with fugue.

L14 ANSWER 11 OF 23 MEDLINE on STN
ACCESSION NUMBER: 1998445375 MEDLINE
DOCUMENT NUMBER: 98445375 PubMed ID: 9770490
TITLE: Nuclear-encoded proteins target to the plastid in Toxoplasma gondii and Plasmodium falciparum.
AUTHOR: Waller R F; Keeling P J; Donald R G; Striepen B; Handman E; Lang-Unnasch N; Cowman A F; Besra G S; Roos D S; McFadden G I
CORPORATE SOURCE: Plant Cell Biology Research Centre, School of Botany,

University of Melbourne, Parkville VIC 3052, Australia.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Oct 13) 95 (21) 12352-7.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF038922; GENBANK-AF038923; GENBANK-AF038924; GENBANK-AF038925; GENBANK-AF038926; GENBANK-AF038927; GENBANK-AF038928; GENBANK-AF038929; GENBANK-AF067150
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981112

AB A vestigial, nonphotosynthetic plastid has been identified recently in protozoan parasites of the phylum Apicomplexa. The apicomplexan plastid, or "apicoplast," is indispensable, but the complete sequence of both the Plasmodium falciparum and Toxoplasma gondii apicoplast genomes has offered no clue as to what essential metabolic function(s) this organelle might perform in parasites. To investigate possible functions of the apicoplast, we sought to identify nuclear-encoded genes whose products are targeted to the apicoplast in Plasmodium and Toxoplasma. We describe here nuclear genes encoding ribosomal proteins S9 and L28 and the fatty acid biosynthetic enzymes **acyl carrier protein** (ACP), beta-ketoacyl-ACP synthase III (FabH), and beta-hydroxyacyl-ACP dehydratase (FabZ). These genes show high similarity to plastid homologues, and immunolocalization of S9 and ACP verifies that the proteins accumulate in the plastid. All the putatively apicoplast-targeted proteins bear N-terminal presequences consistent with plastid targeting, and the ACP presequence is shown to be sufficient to target a recombinant green **fluorescent** protein reporter to the apicoplast in transgenic T. gondii. Localization of ACP, and very probably FabH and FabZ, in the apicoplast implicates fatty acid biosynthesis as a likely function of the apicoplast. Moreover, inhibition of P. falciparum growth by thiolactomycin, an inhibitor of FabH, indicates a vital role for apicoplast fatty acid biosynthesis. Because the fatty acid biosynthesis genes identified here are of a plastid/bacterial type, and distinct from those of the equivalent pathway in animals, fatty acid biosynthesis is potentially an excellent target for therapeutics directed against malaria, toxoplasmosis, and other apicomplexan-mediated diseases.

L14 ANSWER 12 OF 23 MEDLINE on STN
ACCESSION NUMBER: 87008599 MEDLINE
DOCUMENT NUMBER: 87008599 PubMed ID: 3531208
TITLE: Fluorescence studies of chicken liver fatty acid synthase. Segmental flexibility and distance measurements.
AUTHOR: Yuan Z Y; Hammes G G
CONTRACT NUMBER: GM 13292 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1986 Oct 15) 261 (29) 13643-51.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198611
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19970203
Entered Medline: 19861117

AB The 4'-phosphopantetheine of chicken liver fatty acid synthase was specifically labeled with the **fluorescent** substrate analog coenzyme A 6-[7-nitrobenz-2-oxa-1,3-diazol-4-yl]aminohexanoate at low salt concentrations. A serine at the active site of the thioesterase was specifically labeled with the **fluorescent** compounds

6-[7-nitrobenz-2-oxa-1,3-diazol-4-yl]aminopentylmethylphosphono fluoridate and/or pyrenebutyl methylphosphonofluoridate. Dynamic anisotropy measurements indicate the thioesterase has considerable segmental flexibility, whereas the **fluorescent** labeled 4'-phosphopantetheine does not display detectable local or segmental flexibility. Fluorescence resonance energy transfer measurements indicate that the distance between the **fluorescent** label at the end of the 4'-phosphopantetheine and NADPH bound to the beta-ketoacyl reductase or enoyl reductase site on the same polypeptide chain is essentially the same, approximately 38 A. The two types of reductases were distinguished by specifically blocking enoyl reductase with pyridoxal 5'-phosphate. No significant energy transfer occurs between sites on different polypeptide chains so that the distances must be greater than 55 A. The distance between the serine on the thioesterase and the 4'-phosphopantetheine on the same polypeptide is 48 A; again no interpolypeptide chain energy transfer was observed. The distance between the serines of the two thioesterases within a fatty acid synthase molecule is greater than 56 A. The monomeric enzyme obtained at 1 degree C does not have beta-ketoacyl synthase and reductase activities. Also **fluorescent** titrations indicate NADPH is not bound to beta-ketoacyl reductase in monomeric enzyme. The addition of potassium phosphate to the monomers at 1 degree C rapidly dimerizes the enzyme and restores the beta-ketoacyl reductase activity. The beta-ketoacyl synthase activity is slowly restored when the dimer is raised to room temperature. The results obtained suggest that relatively large conformational changes may be part of the catalytic cycle.

L14 ANSWER 13 OF 23 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 93:16665 SCISEARCH
THE GENUINE ARTICLE: KE777
TITLE: THE ROLE OF FATTY-ACID BIOSYNTHESIS AND DEGRADATION IN THE SUPPLY OF SUBSTRATES FOR POLY(3-HYDROXYALKANOATE) FORMATION IN PSEUDOMONAS-PUTIDA
AUTHOR: EGGINK G (Reprint); DEWAARD P; HUIJBERTS G N M
CORPORATE SOURCE: ATO DLO, POB 17, 6700 AA WAGENINGEN, NETHERLANDS (Reprint)
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: FEMS MICROBIOLOGY REVIEWS, (DEC 1992) Vol. 103, No. 2-4, pp. 159-163.
ISSN: 0168-6445.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 11
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The relationship between fatty acid metabolism and PHA biosynthesis in P. putida is described. Detailed H-1 and C-13 NMR studies were performed to investigate the structures of poly(3-hydroxyalkanoates) (PHAs) formed from carbohydrates and fatty acids. On the basis of these results, it is proposed that during growth on glucose the 3-hydroxyacyl-**acyl carrier protein** intermediates of the de novo fatty acid biosynthetic pathway are diverted to PHA biosynthesis. Similarly, further evidence is presented that during cultivation on fatty acids, intermediates of the beta-oxidation cycle serve as precursors of PHA biosynthesis.

L14 ANSWER 14 OF 23 MEDLINE on STN
ACCESSION NUMBER: 86077793 MEDLINE
DOCUMENT NUMBER: 86077793 PubMed ID: 4074750
TITLE: Mapping of acyl carrier domain within the subunit of type I bacterial fatty acid synthetase.
AUTHOR: Morishima N; Ikai A
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1985 Dec 20) 832 (3) 297-307.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198602
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860212

AB A **fluorescent** thiol reagent, N-(7-dimethylamino-4-methylcoumarinyl) maleimide, was used to label the acyl carrier site of the bacterial fatty acid synthetase from Brevibacterium ammoniagenes. The reagent bound preferentially to the 4'-phosphopantetheine thiol group of the acyl carrier domain and irreversively inactivated the enzyme. The modified enzyme was cleaved by proteinases for the mapping of the labeled site. The **fluorescent** fragment was readily detected on a polyacrylamide gel after electrophoresis. The region of 45 kDa containing the 4'-phosphopantetheine was located on the polypeptide at around two-thirds of the full length from the N-terminal.

L14 ANSWER 15 OF 23 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003450401 EMBASE
TITLE: Heterologous expression of hybrid type II polyketide synthase system in Streptomyces species.
AUTHOR: Kim C.-Y.; Park H.-J.; Kim E.-S.
CORPORATE SOURCE: E.-S. Kim, School of Chem. Eng./Biotechnology, Inha University, Incheon 402-751, Korea, Republic of. eungsoo@inha.ac.kr
SOURCE: Journal of Microbiology and Biotechnology, (2003) 13/5 (819-822).
Refs: 23
ISSN: 1017-7825 CODEN: JOMBES
COUNTRY: Korea, Republic of
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Polyketides are an extensive class of secondary metabolites with diverse molecular structures and biological activities. A plasmid-based minimal polyketide synthase (PKS) expression cassette was constructed using a subset of actinorhodin (act) biosynthetic genes (actI-orf1, actI-orf2, actI-orf3, actIII, actVII, and actIV) from Streptomyces coelicolor, which specify the construction of an orange-**fluorescent** anthraquinone product aloesaponarin II, a type II polyketide compound derived from one acetyl coenzyme A and 7 malonyl coenzyme A extender units. This system was designed as an indicator pathway in S. parvulus to generate a hybrid type II polyketide compound via gene-specific replacement. The act .beta.-ketoacyl synthase unit (actI-orf1 and actI-orf2) in the expression cassette was specifically replaced with oxytetracycline .beta.-ketoacyl synthase (otcY-orf1 and otcY-orf2). This plasmid-based hybrid PKS cassette generated a novel orange-**fluorescent** compound structurally different from aloesaponarin II in both S. lividans and S. parvulus. In addition, several additional distinctive blue-**fluorescent** compounds were detected, when this hybrid PKS cassette was expressed in S. coelicolor B78 (actI-orf2 mutant), implying that the expression of plasmid-based hybrid PKS cassette in Streptomyces species should be an efficient way of generating hybrid type II polyketide compounds.

L14 ANSWER 16 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2003448010 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12946854
TITLE: Targeting GFP to the malarial mitochondrion.
AUTHOR: Sato Shigeharu; Rangachari Kaveri; Wilson R J M
CORPORATE SOURCE: Division of Parasitology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK..

ssato@nimr.mrc.ac.uk
SOURCE: Molecular and biochemical parasitology, (2003 Aug 31) 130 (2) 155-8.
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20030928
Last Updated on STN: 20040107
Entered Medline: 20040106

L14 ANSWER 17 OF 23 MEDLINE on STN
ACCESSION NUMBER: 84032436 MEDLINE
DOCUMENT NUMBER: 84032436 PubMed ID: 6630195
TITLE: The arrangement and role of some of the amino acid residues in the beta-ketoacyl synthetase site of chicken liver fatty acid synthetase.
AUTHOR: Stoops J K; Henry S J; Wakil S J
CONTRACT NUMBER: AM21286 (NIADDK)
GM 19091 (NIGMS)
HL17269 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1983 Oct 25) 258 (20) 12482-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198312
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 20000303
Entered Medline: 19831217

AB The beta-ketoacyl synthetase site of eukaryotic fatty acid synthetases is comprised in part of a pantetheinyl residue on one subunit juxtapositioned with a cysteinyl residue on the adjacent subunit. The present study has confirmed this arrangement and has identified 2 additional residues in the site. The active site residues were identified as summarized below. Sodium borohydride reduction of the keto derivatives of the dibromopropanone cross-linked residues yielded the alcohol derivatives which were amenable to isolation in good yields. The active enzyme yielded primarily a cysteinecysteamine derivative of 2-propanol, demonstrating that a cystyl and the pantetheinyl residues were cross-linked by dibromopropanone. However, in the cold-inactivated enzyme, the primary product of the cross-linking reaction was the dicystyl derivative. In addition, cross-linking between the cystyl and pantetheinyl residues, but not the two cystyl residues, resulted in the cross-linking of the two subunits. Therefore, it is proposed that there are two cystyl residues on one subunit juxtapositioned with the pantetheinyl residue on the adjacent subunit. The cystyl residues are highly reactive toward alkylating agents at pH 6.5, suggesting the presence of a cationic residue interacting with the thiolate anion. This proposal was supported using the bifunctional reagent o-phthalaldehyde which was found to cross-link the epsilon-amino group of lysine with the pantetheinyl-SH or the cystyl-SH in the beta-ketoacyl synthetase site to form a thioisoindole ring. The dialdehyde inhibited the enzyme by inactivating the beta-ketoacyl synthetase activity, and the inhibition could be prevented by malonyl-CoA and to a lesser extent by acetyl-CoA. Blocking the reactive thiol groups with dibromopropanone or 5,5'-dithiobis(2-nitrobenzoic acid) reduced the formation of the **fluorescent** thioisoindole ring. The close arrangement of a cystyl-SH, the pantetheinyl-SH, and the epsilon-amino group of lysine led us to propose that the positive epsilon-amino group may serve as an electron sink in a general acid-catalyzed decarboxylation reaction.

L14 ANSWER 18 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2002080372 MEDLINE
DOCUMENT NUMBER: 21665472 PubMed ID: 11807257
TITLE: Crystallization and preliminary X-ray crystallographic analysis of the Rv2002 gene product from Mycobacterium tuberculosis, a beta-ketoacyl carrier protein reductase homologue.
AUTHOR: Yang Jin Kuk; Yoon Hye-Jin; Ahn Hyung Jun; Lee Byung Il; Cho Sang Hyun; Waldo Geoffrey S; Park Min S; Suh Se Won
CORPORATE SOURCE: Structural Proteomics Laboratory, School of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-742, South Korea.
SOURCE: ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (2002 Feb) 58 (Pt 2) 303-5. Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020128
Last Updated on STN: 20020602
Entered Medline: 20020531

AB A 260-residue protein (FabG3) encoded by the Rv2002 gene of Mycobacterium tuberculosis shows amino-acid sequence similarity to beta-ketoacyl carrier protein (ACP) reductase, FabG. A soluble mutant (I6T/V47M/T69M) was produced by the green **fluorescent** protein-based directed-evolution method. It was crystallized at 296 K using the hanging-drop vapour-diffusion method. The diffraction quality of the crystal improved significantly after annealing/dehydration. X-ray diffraction data were collected to 1.8 A resolution using synchrotron radiation. The crystal belongs to the space group P3(1)21 (or P3(2)21), with unit-cell parameters a = b = 70.38, c = 148.93 A. The asymmetric unit contains two subunits, with a corresponding V(M) of 1.90 A(3) Da(-1) and a solvent content of 35.3%.

L14 ANSWER 19 OF 23 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:147068 BIOSIS
DOCUMENT NUMBER: PREV200100147068
TITLE: A plastid segregation defect in the protozoan parasite Toxoplasma gondii.
AUTHOR(S): He, Cynthia Y.; Shaw, Michael K.; Pletcher, Charles H.; Striepen, Boris; Tilney, Lewis G.; Roos, David S. [Reprint author]
CORPORATE SOURCE: Department of Biology, University of Pennsylvania, 305 Goddard Laboratories, Philadelphia, PA, 19104, USA droos@mail.sas.upenn.edu
SOURCE: EMBO (European Molecular Biology Organization) Journal, (February 1, 2001) Vol. 20, No. 3, pp. 330-339. print. CODEN: EMJODG. ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Mar 2001
Last Updated on STN: 15 Feb 2002

AB Apicomplexan parasites-including the causative agents of malaria (Plasmodium sp.) and toxoplasmosis (Toxoplasma gondii)-harbor a secondary endosymbiotic plastid, acquired by lateral genetic transfer from a eukaryotic alga. The apicoplast has attracted considerable attention, both as an evolutionary novelty and as a potential target for chemotherapy. We report a recombinant fusion (between a nuclear-encoded apicoplast protein, the green **fluorescent** protein and a rhoptry protein) that targets to the apicoplast but grossly alters its morphology, preventing organellar segregation during parasite division. Apicoplast-deficient parasites replicate normally in the first infectious

cycle and can be isolated by fluorescence-activated cell sorting, but die in the subsequent host cell, confirming the 'delayed death' phenotype previously described pharmacologically, and validating the apicoplast as essential for parasite viability.

L14 ANSWER 20 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2003051019 MEDLINE
DOCUMENT NUMBER: 22448298 PubMed ID: 12560551
TITLE: Dissecting apicoplast targeting in the malaria parasite Plasmodium falciparum.
AUTHOR: Foth Bernardo J; Ralph Stuart A; Tonkin Christopher J; Struck Nicole S; Fraunholz Martin; Roos David S; Cowman Alan F; McFadden Geoffrey I
CORPORATE SOURCE: Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, VIC 3010, Australia.
SOURCE: SCIENCE, (2003 Jan 31) 299 (5607) 705-8.
Journal code: 0404511. ISSN: 1095-9203.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20030202
Last Updated on STN: 20030222
Entered Medline: 20030221

AB Transit peptides mediate protein targeting into plastids and are only poorly understood. We extracted amino acid features from transit peptides that target proteins to the relict plastid (apicoplast) of malaria parasites. Based on these amino acid characteristics, we identified 466 putative apicoplast proteins in the Plasmodium falciparum genome. Altering the specific charge characteristics in a model transit peptide by site-directed mutagenesis severely disrupted organellar targeting in vivo. Similarly, putative Hsp70 (DnaK) binding sites present in the transit peptide proved to be important for correct targeting.

L14 ANSWER 21 OF 23 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2003:476238 SCISEARCH
THE GENUINE ARTICLE: 682EH
TITLE: Characterization of 2-enoyl thioester reductase from mammals - An ortholog of Ybr026p/Mrf1 ' p of the yeast mitochondrial fatty acid synthesis type II
AUTHOR: Miinalainen I J; Chen Z J; Torkko J M; Pirila P L; Sormunen R T; Bergmann U; Qin Y M; Hiltunen J K (Reprint)
CORPORATE SOURCE: Univ Oulu, Dept Biochem, Bioctr Oulu, PO 3000, FIN-90014 Oulu, Finland (Reprint); Univ Oulu, Dept Biochem, Bioctr Oulu, FIN-90014 Oulu, Finland; Univ Oulu, Dept Pathol, FIN-90014 Oulu, Finland; Ruhr Univ Bochum, Inst Physiol Chem, D-44780 Bochum, Germany
COUNTRY OF AUTHOR: Finland; Germany
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (30 MAY 2003) Vol. 278, No. 22, pp. 20154-20161.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 50

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A data base search with YBR026c/MRF1', which encodes trans-2-enoyl thioester reductase of the intramitochondrial fatty acid synthesis (FAS) type II in yeast (Torkko, J.M., Koivuranta, K.T., Miinalainen, I.J., Yagi, A.I., Schmitz, W., Kastaniotis, A.J., Airenne, T.T., Gurvitz, A., and Hiltunen, K.J. (2001) Mol. Cell. Biol. 21, 6243-6253), revealed the clone AA393871 (HsNrbf-1, nuclear receptor binding factor 1) in human EST data bank. Expression of HsNrbf-1, tagged C-terminally with green

fluorescent protein, in HeLa cells, resulted in a punctated fluorescence signal, superimposable with the MitoTracker Red dye. Wild-type polypeptide was immunoisolated from the extract of bovine heart mitochondria. Recombinant HsNrbf-1p reduces trans-2-enoyl-CoA to acyl-CoA with chain length from C6 to C16 in an NADPH-dependent manner with preference to medium chain length substrate. Furthermore, expression of HsNRBF-1 in the ybr026cDelta yeast strain restored mitochondrial respiratory function allowing growth on glycerol. These findings provide evidence that Nrbf-1ps act as a mitochondrial 2-enoyl thioester reductase, and mammalian cells may possess bacterial type fatty acid synthetase (FAS type II) in mitochondria, in addition to FAS type I in the cytoplasm.

L14 ANSWER 22 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:489673 CAPLUS
DOCUMENT NUMBER: 135:87150
TITLE: High throughput screen for inhibitors of fatty acid biosynthesis in bacteria
INVENTOR(S): Murphy, Christopher; Youngman, Philip
PATENT ASSIGNEE(S): Millennium Pharmaceuticals Inc., USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001048248	A2	20010705	WO 2000-US35598	20001229
WO 2001048248	A3	20020919		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 6656703	B1	20031202	US 1999-474140	19991229

PRIORITY APPLN. INFO.: US 1999-474140 A1 19991229
AB Methods for identifying compds. that are inhibitors of bacterial fatty acid biosynthesis are disclosed. Such compds. can be used as lead compds. in methods for prepg. antibacterial agents for treating bacterial infections (e.g., in humans, animals, and plants). Inhibitors of bacterial fatty acid synthesis can also be tested for their ability to inhibit synthesis of acylated homoserine lactones. Compds. that inhibit synthesis of acylated homoserine lactones can be used as inhibitors of bacterial virulence. The disclosed methods allow for high throughput screening of libraries of test compds.

L14 ANSWER 23 OF 23 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:164350 BIOSIS
DOCUMENT NUMBER: PREV200200164350
TITLE: Organellar segregation mutants validate the apicomplexan plastid as essential for survival of the protozoan parasite Toxoplasma gondii.
AUTHOR(S): He, Cynthia Y. [Reprint author]; Shaw, Michael K. [Reprint author]; Striepen, Boris; Pletcher, Hank [Reprint author]; Roos, David S.
CORPORATE SOURCE: University of Pennsylvania, 415 South University Avenue, Philadelphia, PA, 19104-6018, USA
SOURCE: Molecular Biology of the Cell, (Dec., 2000) Vol. 11, No. Supplement, pp. 527a-528a. print.
Meeting Info.: 40th American Society for Cell Biology

Annual Meeting. San Francisco, CA, USA. December 09-13, 2000. American Society for Cell Biology.
CODEN: MBCEEV. ISSN: 1059-1524.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Mar 2002
Last Updated on STN: 5 Mar 2002

=> s (acyl carrier protein or apo-acyl carrier protein or holo-acyl carrier protein or a
5 FILES SEARCHED...
L15 20 (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACYL CARRIER PROTEIN OR ACYLATED ACYL CARRIER PROTEIN) AND GREEN FLUORESCENT

=> dup rem l15
PROCESSING COMPLETED FOR L15
L16 10 DUP REM L15 (10 DUPLICATES REMOVED)

=> focus l16
PROCESSING COMPLETED FOR L16
L17 10 FOCUS L16 1-

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L17 ANSWER 1 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2003466211 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12882974
TITLE: Cloning, expression, characterization, and interaction of two components of a human mitochondrial fatty acid synthase. Malonyltransferase and **acyl carrier protein.**
AUTHOR: Zhang Lei; Joshi Anil K; Smith Stuart
CORPORATE SOURCE: Children's Hospital Oakland Research Institute, Oakland, California 94609.
CONTRACT NUMBER: DK 16073 (NIDDK)
SOURCE: Journal of biological chemistry, (2003 Oct 10) 278 (41) 40067-74.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200312
ENTRY DATE: Entered STN: 20031008
Last Updated on STN: 20031218
Entered Medline: 20031202

AB The possibility that human cells contain, in addition to the cytosolic type I fatty acid synthase complex, a mitochondrial type II malonyl-CoA-dependent system for the biosynthesis of fatty acids has been examined by cloning, expressing, and characterizing two putative components. Candidate coding sequences for a malonyl-CoA:**acyl carrier protein** transacylase (malonyltransferase) and its **acyl carrier protein** substrate, identified by BLAST searches of the human sequence data base, were located on nuclear chromosomes 22 and 16, respectively. The encoded proteins localized exclusively in mitochondria only when the putative N-terminal mitochondrial targeting sequences were present as revealed by confocal microscopy of HeLa cells infected with appropriate **green fluorescent** protein fusion constructs. The mature, processed forms of the mitochondrial proteins were expressed in Sf9 cells and purified, the **acyl carrier protein** was converted to the holoform in vitro using purified human phosphopantetheinyltransferase, and the functional interaction of the two

proteins was studied. Compared with the dual specificity malonyl/acetyltransferase component of the cytosolic type I fatty acid synthase, the type II mitochondrial counterpart exhibits a relatively narrow substrate specificity for both the acyl donor and **acyl carrier protein** acceptor. Thus, it forms a covalent acyl-enzyme complex only when incubated with malonyl-CoA and transfers exclusively malonyl moieties to the mitochondrial holoacyl carrier protein. The type II **acyl carrier protein** from Bacillus subtilis, but not the **acyl carrier protein** derived from the human cytosolic type I fatty acid synthase, can also function as an acceptor for the mitochondrial transferase. These data provide compelling evidence that human mitochondria contain a malonyl-CoA/**acyl carrier protein**-dependent fatty acid synthase system, distinct from the type I cytosolic fatty acid synthase, that resembles the type II system present in prokaryotes and plastids. The final products of this system, yet to be identified, may play an important role in mitochondrial function.

L17 ANSWER 2 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2003595287 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 14675432
TITLE: Organisation of the pantothenate (vitamin B5) biosynthesis pathway in higher plants.
AUTHOR: Ottenhof Harald H; Ashurst Jennifer L; Whitney Heather M; Saldanha S Adrian; Schmitzberger Florian; Gweon Hyun Soon; Blundell Tom L; Abell Chris; Smith Alison G
CORPORATE SOURCE: Department of Plant Sciences, Downing Street, Cambridge CB2 3EA, UK, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK, and Department of Biochemistry, 80 Tennis Court Road, Cambridge CB2 1GA, UK.
SOURCE: Plant journal : for cell and molecular biology, (2004 Jan) 37 (1) 61-72.
Journal code: 9207397. ISSN: 0960-7412.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20031217
Last Updated on STN: 20031217

AB Pantothenate (vitamin B5) is the precursor for the biosynthesis of the phosphopantetheine moiety of coenzyme A and **acyl carrier protein**, and is synthesised in Escherichia coli by four enzymic reactions. Ketopantoate hydroxymethyltransferase (KPHMT) and pantothenate synthetase (PtS) catalyse the first and last steps, respectively. Two genes encoding KPHMT and one for PtS were identified in the Arabidopsis thaliana genome, and cDNAs for all three genes were amplified by PCR. The cDNAs were able to complement their respective E. coli auxotrophs, demonstrating that they encoded functional enzymes. Subcellular localisation of the proteins was investigated using **green fluorescent** protein (GFP) fusions and confocal microscopy. The two KPHMT-GFP fusion proteins were targeted exclusively to mitochondria, whereas PtS-GFP was found in the cytosol. This implies that there must be transporters for pathway intermediates. KPHMT enzyme activity could be measured in purified mitochondria from both pea leaves and Arabidopsis suspension cultures. We investigated whether Arabidopsis encoded homologues of the remaining two pantothenate biosynthesis enzymes from E. coli, l-aspartate-alpha-decarboxylase (ADC) and ketopantoate reductase (KPR). No homologue of ADC could be identified using either conventional blast or searches with the program fugue in which the structure of the E. coli ADC was compared to all the annotated proteins in Arabidopsis. ADC also appears to be absent from the genome of the yeast, Saccharomyces cerevisiae, by the same criteria. In contrast, a putative Arabidopsis oxidoreductase with some similarity to KPR was identified with fugue.

L17 ANSWER 3 OF 10 MEDLINE on STN
ACCESSION NUMBER: 1998445375 MEDLINE
DOCUMENT NUMBER: 98445375 PubMed ID: 9770490
TITLE: Nuclear-encoded proteins target to the plastid in Toxoplasma gondii and Plasmodium falciparum.
AUTHOR: Waller R F; Keeling P J; Donald R G; Striepen B; Handman E; Lang-Unnasch N; Cowman A F; Besra G S; Roos D S; McFadden G I
CORPORATE SOURCE: Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville VIC 3052, Australia.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Oct 13) 95 (21) 12352-7. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF038922; GENBANK-AF038923; GENBANK-AF038924; GENBANK-AF038925; GENBANK-AF038926; GENBANK-AF038927; GENBANK-AF038928; GENBANK-AF038929; GENBANK-AF067150
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981112

AB A vestigial, nonphotosynthetic plastid has been identified recently in protozoan parasites of the phylum Apicomplexa. The apicomplexan plastid, or "apicoplast," is indispensable, but the complete sequence of both the Plasmodium falciparum and Toxoplasma gondii apicoplast genomes has offered no clue as to what essential metabolic function(s) this organelle might perform in parasites. To investigate possible functions of the apicoplast, we sought to identify nuclear-encoded genes whose products are targeted to the apicoplast in Plasmodium and Toxoplasma. We describe here nuclear genes encoding ribosomal proteins S9 and L28 and the fatty acid biosynthetic enzymes **acyl carrier protein** (ACP), beta-ketoacyl-ACP synthase III (FabH), and beta-hydroxyacyl-ACP dehydratase (FabZ). These genes show high similarity to plastid homologues, and immunolocalization of S9 and ACP verifies that the proteins accumulate in the plastid. All the putatively apicoplast-targeted proteins bear N-terminal presequences consistent with plastid targeting, and the ACP presequence is shown to be sufficient to target a recombinant **green fluorescent** protein reporter to the apicoplast in transgenic T. gondii. Localization of ACP, and very probably FabH and FabZ, in the apicoplast implicates fatty acid biosynthesis as a likely function of the apicoplast. Moreover, inhibition of P. falciparum growth by thiolactomycin, an inhibitor of FabH, indicates a vital role for apicoplast fatty acid biosynthesis. Because the fatty acid biosynthesis genes identified here are of a plastid/bacterial type, and distinct from those of the equivalent pathway in animals, fatty acid biosynthesis is potentially an excellent target for therapeutics directed against malaria, toxoplasmosis, and other apicomplexan-mediated diseases.

L17 ANSWER 4 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2002080372 MEDLINE
DOCUMENT NUMBER: 21665472 PubMed ID: 11807257
TITLE: Crystallization and preliminary X-ray crystallographic analysis of the Rv2002 gene product from Mycobacterium tuberculosis, a beta-ketoacyl carrier protein reductase homologue.
AUTHOR: Yang Jin Kuk; Yoon Hye-Jin; Ahn Hyung Jun; Lee Byung Il; Cho Sang Hyun; Waldo Geoffrey S; Park Min S; Suh Se Won
CORPORATE SOURCE: Structural Proteomics Laboratory, School of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-742, South Korea.
SOURCE: ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (2002 Feb) 58 (Pt 2) 303-5.

Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020128
Last Updated on STN: 20020602
Entered Medline: 20020531

AB A 260-residue protein (FabG3) encoded by the Rv2002 gene of Mycobacterium tuberculosis shows amino-acid sequence similarity to beta-ketoacyl carrier protein (ACP) reductase, FabG. A soluble mutant (I6T/V47M/T69M) was produced by the **green fluorescent** protein-based directed-evolution method. It was crystallized at 296 K using the hanging-drop vapour-diffusion method. The diffraction quality of the crystal improved significantly after annealing/dehydration. X-ray diffraction data were collected to 1.8 A resolution using synchrotron radiation. The crystal belongs to the space group P3(1)21 (or P3(2)21), with unit-cell parameters a = b = 70.38, c = 148.93 A. The asymmetric unit contains two subunits, with a corresponding V(M) of 1.90 A(3) Da(-1) and a solvent content of 35.3%.

L17 ANSWER 5 OF 10 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:147068 BIOSIS
DOCUMENT NUMBER: PREV200100147068
TITLE: A plastid segregation defect in the protozoan parasite Toxoplasma gondii.
AUTHOR(S): He, Cynthia Y.; Shaw, Michael K.; Pletcher, Charles H.; Striepen, Boris; Tilney, Lewis G.; Roos, David S. [Reprint author]
CORPORATE SOURCE: Department of Biology, University of Pennsylvania, 305 Goddard Laboratories, Philadelphia, PA, 19104, USA droos@mail.sas.upenn.edu
SOURCE: EMBO (European Molecular Biology Organization) Journal, (February 1, 2001) Vol. 20, No. 3, pp. 330-339. print. CODEN: EMJODG. ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Mar 2001
Last Updated on STN: 15 Feb 2002

AB Apicomplexan parasites-including the causative agents of malaria (Plasmodium sp.) and toxoplasmosis (Toxoplasma gondii)-harbor a secondary endosymbiotic plastid, acquired by lateral genetic transfer from a eukaryotic alga. The apicoplast has attracted considerable attention, both as an evolutionary novelty and as a potential target for chemotherapy. We report a recombinant fusion (between a nuclear-encoded apicoplast protein, the **green fluorescent** protein and a rhoptry protein) that targets to the apicoplast but grossly alters its morphology, preventing organellar segregation during parasite division. Apicoplast-deficient parasites replicate normally in the first infectious cycle and can be isolated by fluorescence-activated cell sorting, but die in the subsequent host cell, confirming the 'delayed death' phenotype previously described pharmacologically, and validating the apicoplast as essential for parasite viability.

=> s acyl carrier protein and (dansyl or fluorescein or rhodamine or FITC or TRITC or Te
L18 13 ACYL CARRIER PROTEIN AND (DANSYL OR FLUORESCEIN OR RHODAMINE OR FITC OR TRITC OR TEXAS RED)

=> dup rem l18
PROCESSING COMPLETED FOR L18
L19 6 DUP REM L18 (7 DUPLICATES REMOVED)

=> d l19 1-6 ibib ab

L19 ANSWER 1 OF 6 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002702655 MEDLINE
DOCUMENT NUMBER: 22351773 PubMed ID: 12463745
TITLE: Fluorescence anisotropy studies of enzyme-substrate complex formation in stearoyl-ACP desaturase.
AUTHOR: Haas Jeffrey A; Fox Brian G
CORPORATE SOURCE: Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706, USA.
CONTRACT NUMBER: GM-50853 (NIGMS)
SOURCE: BIOCHEMISTRY, (2002 Dec 10) 41 (49) 14472-81.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021217
Last Updated on STN: 20030125
Entered Medline: 20030124

AB Stearoyl-**acyl carrier protein** Delta(9)-desaturase (delta9D) catalyzes regio- and stereospecific insertion of cis double bonds into acyl chains attached to **acyl carrier protein**. Steady-state and stopped-flow fluorescence anisotropy measurements using acylated forms of **dansyl**- and fluoresceinyl-ACPs revealed equilibrium dissociation constants and dissociation rate constants for 16:0-, 17:0-, and 18:0-ACPs with resting and chemically 4e(-) reduced delta9D. Binding of 1 nM 18:0-fluoresceinyl-ACP to one subunit of the dimeric resting delta9D was observed with K(D1) = 13 +/- 3 nM. No significant difference in the K(D1) value was observed for 4e(-) delta9D. An approximately 4-fold increase in K(D1) per methylene group was observed upon shortening the acyl chain from 18:0 to 17:0 and then 16:0. In different experiments performed with 850 nM 18:0-**dansyl**-ACP, binding to the second subunit of resting delta9D was estimated to have K(D2) approximately 350 +/- 40 nM. The K(D2) values exhibited a similar dependence on acyl chain length as observed for the K(D1) values. The k(off) values measured by stopped-flow anisotropy measurements for reversal of the enzyme-substrate complex were also acyl-chain length dependent and increased 130-fold for 16:0-ACP (130 s(-)(1)) relative to 18:0-ACP (1 s(-)(1)). Increases in acyl chain length are thus associated with the presently reported increases in the K(D) and k(off) values. These results indicate that acyl chain length selectivity derives in major part from partition of the enzyme-substrate complex between substrate release and subsequent steps in catalysis.

L19 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:513782 CAPLUS
DOCUMENT NUMBER: 133:132135
TITLE: Preparation of genetically modified proteins for site-specific transglutaminase-mediated labeling and applications in high-throughput screening
INVENTOR(S): Tew, David G.; Powell, David J.; Meek, Thomas D.; Chen, Wenfang
PATENT ASSIGNEE(S): Smithkline Beecham Corporation, USA
SOURCE: PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000043492	A2	20000727	WO 2000-US1481	20000120

W: JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
EP 1151299 A1 20011107 EP 2000-911605 20000120
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
JP 2003527561 T2 20030916 JP 2000-594901 20000120
PRIORITY APPLN. INFO.: US 1999-117327P P 19990122
WO 2000-US1481 W 20000120

AB Methods for site-specific modification of protein are provided. These methods modify proteins which have been labeled at a particular site by the reaction of a transglutaminase with a glutamine peptide sequence which has been engineered into the protein. The site-specific modification methods of the invention are useful for producing reagents useful in high throughput screening methods and in producing protein delivery vehicles for specifically targeting cellular and non-cellular targets. Also described are improved biotinylation reagents.

L19 ANSWER 3 OF 6 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001073203 MEDLINE
DOCUMENT NUMBER: 20504166 PubMed ID: 11049751
TITLE: Chemical and posttranslational modification of Escherichia coli **acyl carrier protein** for preparation of **dansyl**-acyl carrier proteins.
AUTHOR: Haas J A; Frederick M A; Fox B G
CORPORATE SOURCE: Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, USA.
CONTRACT NUMBER: GM50853 (NIGMS)
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (2000 Nov) 20 (2) 274-84.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010103

AB Escherichia coli **acyl carrier protein** (ACP) contains a single tyrosine residue at position 71. The combined o-nitration of apo-ACP Y71 by tetranitromethane and reduction to 3-aminotyrosyl-apo-ACP were performed to introduce a specific site for attachment of a **dansyl** fluorescent label. Conditions for purification and characterization of dansylaminotyrosyl-apo-ACP are reported. Dansylaminotyrosyl-apo-ACP was enzymatically phosphopantetheinylated and acylated in vitro with an overall approximately 30% yield of purified stearoyl-dansylaminotyrosyl-ACP starting from unmodified apo-ACP. The steady-state kinetic parameters k(cat) = 22 min(-1) and K(M) = 2.7 microM were determined for reaction of stearoyl-dansylaminotyrosyl-ACP with stearoyl-ACP Delta(9)-desaturase. These results show that dansylaminotyrosyl-ACP will function well for studying binding interactions with the Delta(9)-desaturase and suggest similar possibilities for other ACP-dependent enzymes. The efficient in vivo phosphopantetheinylation of E. coli apo-ACP by coexpression with holo-ACP synthase in E. coli BL21(DE3) using fructose as the carbon source is also reported.

L19 ANSWER 4 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:540760 BIOSIS
DOCUMENT NUMBER: PREV200000540760
TITLE: The use of **fluorescein**-labeled Co-enzyme A for the detection of **acyl carrier protein** synthase (AcpS) activity.

AUTHOR(S): McAllister, K. A. [Reprint author]; Richardson, J. M. [Reprint author]; Zhao, G. [Reprint author]
CORPORATE SOURCE: Eli Lilly and Company, Indianapolis, IN, USA
SOURCE: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2000) Vol. 40, pp. 225. print. Meeting Info.: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto, Ontario, Canada. September 17-20, 2000.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Dec 2000
Last Updated on STN: 11 Jan 2002

L19 ANSWER 5 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1999:587953 SCISEARCH
THE GENUINE ARTICLE: 219XP
TITLE: Active/de-active state transition of the mitochondrial complex I as revealed by specific sulfhydryl group labeling
AUTHOR: Gavrikova E V; Vinogradov A D (Reprint)
CORPORATE SOURCE: MOSCOW MV LOMONOSOV STATE UNIV, SCH BIOL, DEPT BIOCHEM, MOSCOW 119899, RUSSIA (Reprint); MOSCOW MV LOMONOSOV STATE UNIV, SCH BIOL, DEPT BIOCHEM, MOSCOW 119899, RUSSIA
COUNTRY OF AUTHOR: RUSSIA
SOURCE: FEBS LETTERS, (16 JUL 1999) Vol. 455, No. 1-2, pp. 36-40. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0014-5793.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 20
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The sensitivities of NADH oxidase and/or NADH-ubiquinone reductase activities of submitochondrial particles and purified complex I towards N-ethylmaleimide (NEM) and other SH-reagents were studied, Only thermally de-activated preparations [A.D. Vinogradov (1998) Biochim, Biophys, Acta 1364, 169-185] were inhibited by SH-reagents whereas the redox-pulsed, activated enzyme was resistant to the inhibitors. The pH profile of the pseudo-first order inhibition rate suggested a pK(a) of about 10 for the de-activation-dependent, NEM-reactive sulfhydryl group, NADH-ubiquinone reductase of activated particles treated with an excess of NEM followed by removal of the inhibitor was still capable of slow reversible active/de-active transition, When active, NEM-treated particles were de-activated and further inhibited by N-**fluorescein** maleimide, specific incorporation of the fluorescence label into low molecular mass polypeptide was evident, Comparison of the specific fluorescence labeling of submitochondrial particles, crude and purified complex I showed that the active/de-active state-dependent SH-group is located in a 15 kDa polypeptide (most likely in the 15 kDa IP subunit of the iron-sulfur protein-containing fraction of complex I), (C) 1999 Federation of European Biochemical Societies.

L19 ANSWER 6 OF 6 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 94079079 EMBASE
DOCUMENT NUMBER: 1994079079
TITLE: Kinetics and specificity of peptide-MHC class II complex displacement reactions.
AUTHOR: De Kroon A.I.P.M.; McConnell H.M.
CORPORATE SOURCE: Department of Chemistry, Stanford University,Stanford, CA 94305, United States
SOURCE: Journal of Immunology, (1994) 152/2 (609-619).

ISSN: 0022-1767 CODEN: JOIMA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The peptide-induced acceleration of the dissociation of pre-formed complexes of the detergent-solubilized mouse class II molecules IE(d) and IE(k) with **fluorescein**-labeled peptides was investigated using high- performance size exclusion chromatography. While it is generally believed that functional complexes of MHC class II .alpha..beta. heterodimers and peptides have a 1:1 stoichiometry, the data provide qualitative as well as quantitative kinetic evidence that the enhancement of the release of one peptide by a second peptide is due to a two-peptide intermediate. Different combinations of peptides were tested for their ability to accelerate each other's release from IE(d). The importance of positive charge for the interaction with IE(d) was confirmed by the finding that not only dynorphin 1-13 but also poly-L- lysine (14-19 mer) and a peptide corresponding to a mitochondrial presequence (net charge +6) efficiently enhance the release of pre-bound peptides. SDS- PAGE analysis revealed that the efficiently displacing peptides do not stabilize the IE(d) .alpha..beta. heterodimer at acidic pH, in contrast to the IE(d)-restricted antigenic peptide HEL 107-116. The data support a mechanism in which the second peptide binds specifically to the pre-formed class II-peptide complex, which, depending on the properties of the peptides involved, leads to the destabilization of the complex and the release of the first peptide.

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=> d his

     (FILE 'HOME' ENTERED AT 11:35:15 ON 15 JAN 2004)

     FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT
     11:35:53 ON 15 JAN 2004
L1             0 S ACYL CARRIER PROTEIN AND NON-RADIOACTIVE LABEL?
L2             0 S ACYL CARRIER PROTEIN AND CHROMO? LABEL?
L3            17 S ACYL CARRIER PROTEIN AND CHROMOPHORE
L4             5 DUP REM L3 (12 DUPLICATES REMOVED)
L5            35 S ACYL CARRIER PROTEIN AND CHEMICAL MODIFICATION
L6            19 DUP REM L5 (16 DUPLICATES REMOVED)
L7            19 FOCUS L6 1-
L8             0 S ACYL CARRIER PROTEIN AND APO-ACYL CARRIER PROTEIN AND HOLO-AC
L9            79 S (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACY
L10           41 DUP REM L9 (38 DUPLICATES REMOVED)
L11           41 FOCUS L10 1-
L12           37 S (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACY
L13           23 DUP REM L12 (14 DUPLICATES REMOVED)
L14           23 FOCUS L13 1-
L15           20 S (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACY
L16           10 DUP REM L15 (10 DUPLICATES REMOVED)
L17           10 FOCUS L16 1-
L18           13 S ACYL CARRIER PROTEIN AND (DANSYL OR FLUORESCEIN OR RHODAMINE
L19            6 DUP REM L18 (7 DUPLICATES REMOVED)

=> s fox g brian/au
L20            0 FOX G BRIAN/AU

=> e l20
E1             4     L2.6/BI
E2             2     L2.7/BI
E3           819 --> L20/BI
E4             1     L20.8/BI
E5            84     L200/BI
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E6          11     L2000/BI
E7           8     L20000/BI
E8          10     L20001/BI
E9           1     L20002/BI
E10          1     L20003/BI
E11          1     L20004/BI
E12          3     L20005/BI

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L21        144 FOX BRIAN G/AU

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PROCESSING COMPLETED FOR L21
L22         89 DUP REM L21 (55 DUPLICATES REMOVED)

=> d l22 1-5

L22  ANSWER 1 OF 89     MEDLINE on STN                  DUPLICATE 1
AN   2003356348     MEDLINE
DN   22705342   PubMed ID: 12820892
TI   Chain cleavage and sulfoxidation of thiastearoyl-ACP upon reaction with
     stearoyl-ACP desaturase.
AU   White Robert D; Fox Brian G
CS   Department of Biochemistry, College of Agricultural and Life Sciences,
     University of Wisconsin, Madison, Wisconsin 53706-1544, USA.
NC   GM-50853 (NIGMS)
     T32 GM08349 (NIGMS)
SO   BIOCHEMISTRY, (2003 Jul 1) 42 (25) 7828-35.
     Journal code: 0370623. ISSN: 0006-2960.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200308
ED   Entered STN: 20030801
     Last Updated on STN: 20030827
     Entered Medline: 20030826

L22  ANSWER 2 OF 89     MEDLINE on STN                  DUPLICATE 2
AN   2003221067     MEDLINE
DN   22627457   PubMed ID: 12741844
TI   Rapid-mix and chemical quench studies of ferredoxin-reduced stearoyl-acyl
     carrier protein desaturase.
AU   Lyle Karen S; Haas Jeffrey A; Fox Brian G
CS   Department of Biochemistry, College of Agricultural and Life Sciences,
     University of Wisconsin, Madison, Wisconsin 53706, USA.
NC   GM-50853 (NIGMS)
     T32 GM-08293 (NIGMS)
SO   BIOCHEMISTRY, (2003 May 20) 42 (19) 5857-66.
     Journal code: 0370623. ISSN: 0006-2960.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200306
ED   Entered STN: 20030514
     Last Updated on STN: 20030621
     Entered Medline: 20030620

L22  ANSWER 3 OF 89     MEDLINE on STN                  DUPLICATE 3
AN   2003155338     MEDLINE
DN   22558519   PubMed ID: 12640145
TI   Insight into the mechanism of aromatic hydroxylation by toluene
     4-monooxygenase by use of specifically deuterated toluene and p-xylene.
AU   Mitchell Kevin H; Rogge Corina E; Gierahn Todd; Fox Brian G
```

CS Department of Biochemistry, University of Wisconsin, Madison, WI 53706-1544, USA.
NC T32 GM08349 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2003 Apr 1) 100 (7) 3784-9.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200305
ED Entered STN: 20030403
Last Updated on STN: 20030523
Entered Medline: 20030522

L22 ANSWER 4 OF 89 MEDLINE on STN DUPLICATE 4
AN 2003083662 MEDLINE
DN 22483128 PubMed ID: 12595730
TI Crystallization and preliminary analysis of native and N-terminal truncated isoforms of toluene-4-monooxygenase catalytic effector protein.
AU Orville Allen M; Studts Joey M; Lountos George T; Mitchell Kevin H; **Fox Brian G**
CS School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA.. allen.orville@chemistry.gatech.edu
SO ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL CRYSTALLOGRAPHY, (2003 Mar) 59 (Pt 3) 572-5.
Journal code: 9305878. ISSN: 0907-4449.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200310
ED Entered STN: 20030222
Last Updated on STN: 20031008
Entered Medline: 20031007

L22 ANSWER 5 OF 89 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:655260 CAPLUS
TI Role of nucleic acid and protein manipulation technologies in high-throughput structural biology efforts
AU Aceti, David J.; Blommel, Paul G.; Endo, Yaeta; **Fox, Brian G.**; Frederick, Ronnie O.; Hegeman, Adrian D.; Jeon, Won Bae; Kimball, Todd L.; Lee, Jason M.; Newman, Craig S.; Peterson, Francis C.; Sawasaki, Tatsuya; Seder, Kory D.; Sussman, Michael R.; Ulrich, Eldon L.; Wrobel, Russell L.; Thao, Sandy; Vinarov, Dmitriy A.; Volkman, Brian F.; Zhao, Qin
CS Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, 53706, USA
SO Biopolymers (2003), Volume 8, 469-496. Editor(s): Steinbuchel, Dr. Alexander; Fahnestock, Dr. Stephen R. Publisher: Wiley-VCH Verlag GmbH, Weinheim, Germany.
CODEN: 69CJVR
DT Conference; General Review
LA English
RE.CNT 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s l22 and acyl carrier protein
L23 20 L22 AND ACYL CARRIER PROTEIN

=> s l23 and label
L24 1 L23 AND LABEL

=> d L24

L24 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:751243 CAPLUS
DN 134:127457
TI Chemical and Posttranslational Modification of Escherichia coli **Acyl Carrier Protein** for Preparation of Dansyl-Acyl Carrier Proteins
AU Haas, Jeffrey A.; Frederick, Melissa A.; **Fox, Brian G.**
CS Department of Biochemistry, College of Agricultrual and Life Sciences, University of Wisconsin, Madison, WI, 53706, USA
SO Protein Expression and Purification (2000), 20(2), 274-284
CODEN: PEXPEJ; ISSN: 1046-5928
PB Academic Press
DT Journal
LA English
RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s Haas A Jeffrey/au
L25 0 HAAS A JEFFREY/AU

=> s Haas Jeffrey A/au
L26 14 HAAS JEFFREY A/AU

=> dup rem l26
PROCESSING COMPLETED FOR L26
L27 7 DUP REM L26 (7 DUPLICATES REMOVED)

=> s l27 and acp
L28 6 L27 AND ACP

=> d l28 1-6 ibib ab

L28 ANSWER 1 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2003221067 MEDLINE
DOCUMENT NUMBER: 22627457 PubMed ID: 12741844
TITLE: Rapid-mix and chemical quench studies of ferredoxin-reduced stearoyl-acyl carrier protein desaturase.
AUTHOR: Lyle Karen S; **Haas Jeffrey A**; Fox Brian G
CORPORATE SOURCE: Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706, USA.
CONTRACT NUMBER: GM-50853 (NIGMS)
T32 GM-08293 (NIGMS)
SOURCE: BIOCHEMISTRY, (2003 May 20) 42 (19) 5857-66.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20030514
Last Updated on STN: 20030621
Entered Medline: 20030620
AB Stearoyl-**ACP** Delta9 desaturase (Delta9D) catalyzes the NADPH- and O(2)-dependent insertion of a cis double bond between the C9 and C10 positions of stearoyl-**ACP** (18:0-**ACP**) to produce oleoyl-**ACP** (18:1-**ACP**). This work revealed the ability of reduced [2Fe-2S] ferredoxin (Fd) to act as a catalytically competent electron donor during the rapid conversion of 18:0-**ACP** into 18:1-**ACP**. Experiments on the order of addition for substrate and reduced Fd showed high conversion of 18:0-**ACP** to 18:1-**ACP** (approximately 95% per Delta9D active site in a single turnover) when 18:0-**ACP** was added prior to reduced Fd. Reactions of the prereduced enzyme-substrate complex with O(2) and the

oxidized enzyme-substrate complex with reduced Fd were studied by rapid-mix and chemical quench methods. For reaction of the prereduced enzyme-substrate complex, an exponential burst phase (k(burst) = 95 s(-1)) of product formation accounted for approximately 90% of the turnover expected for one subunit in the dimeric protein. This rapid phase was followed by a slower phase (k(linear) = 4.0 s(-1)) of product formation corresponding to the turnover expected from the second subunit. For reaction of the oxidized enzyme-substrate complex with excess reduced Fd, a slower, linear rate (k(obsd) = 3.4 s(-1)) of product formation was observed over approximately 1.5 turnovers per Delta9D active site potentially corresponding to a third phase of reaction. An analysis of the deuterium isotope effect on the two rapid-mix reaction sequences revealed only a modest effect on k(burst) ((D)k(burst) approximately 1.5) and k(linear) (D)k(linear) approximately 1.4), indicating C-H bond cleavage does not contribute significantly to the rate-limiting steps of pre-steady-state catalysis. These results were used to assemble and evaluate a minimal kinetic model for Delta9D catalysis.

L28 ANSWER 2 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2002702655 MEDLINE
DOCUMENT NUMBER: 22351773 PubMed ID: 12463745
TITLE: Fluorescence anisotropy studies of enzyme-substrate complex formation in stearoyl-**ACP** desaturase.
AUTHOR: **Haas Jeffrey A**; Fox Brian G
CORPORATE SOURCE: Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706, USA.
CONTRACT NUMBER: GM-50853 (NIGMS)
SOURCE: BIOCHEMISTRY, (2002 Dec 10) 41 (49) 14472-81.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021217
Last Updated on STN: 20030125
Entered Medline: 20030124

AB Stearoyl-acyl carrier protein Delta(9)-desaturase (delta9D) catalyzes regio- and stereospecific insertion of cis double bonds into acyl chains attached to acyl carrier protein. Steady-state and stopped-flow fluorescence anisotropy measurements using acylated forms of dansyl- and fluoresceinyl-ACPs revealed equilibrium dissociation constants and dissociation rate constants for 16:0-, 17:0-, and 18:0-ACPs with resting and chemically 4e(-) reduced delta9D. Binding of 1 nM 18:0-fluoresceinyl-**ACP** to one subunit of the dimeric resting delta9D was observed with K(D1) = 13 +/- 3 nM. No significant difference in the K(D1) value was observed for 4e(-) delta9D. An approximately 4-fold increase in K(D1) per methylene group was observed upon shortening the acyl chain from 18:0 to 17:0 and then 16:0. In different experiments performed with 850 nM 18:0-dansyl-**ACP**, binding to the second subunit of resting delta9D was estimated to have K(D2) approximately 350 +/- 40 nM. The K(D2) values exhibited a similar dependence on acyl chain length as observed for the K(D1) values. The k(off) values measured by stopped-flow anisotropy measurements for reversal of the enzyme-substrate complex were also acyl-chain length dependent and increased 130-fold for 16:0-**ACP** (130 s(-)(1)) relative to 18:0-**ACP** (1 s(-)(1)). Increases in acyl chain length are thus associated with the presently reported increases in the K(D) and k(off) values. These results indicate that acyl chain length selectivity derives in major part from partition of the enzyme-substrate complex between substrate release and subsequent steps in catalysis.

L28 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:751243 CAPLUS

DOCUMENT NUMBER: 134:127457
TITLE: Chemical and Posttranslational Modification of Escherichia coli Acyl Carrier Protein for Preparation of Dansyl-Acyl Carrier Proteins
AUTHOR(S): **Haas, Jeffrey A.**; Frederick, Melissa A.; Fox, Brian G.
CORPORATE SOURCE: Department of Biochemistry, College of Agricultrual and Life Sciences, University of Wisconsin, Madison, WI, 53706, USA
SOURCE: Protein Expression and Purification (2000), 20(2), 274-284
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Escherichia coli acyl carrier protein (**ACP**) contains a single tyrosine residue at position 71. The combined o-nitration of apo-**ACP** Y71 by tetranitromethane and redn. to 3-aminotyrosyl-apo-**ACP** were performed to introduce a specific site for attachment of a dansyl fluorescent label. Conditions for purifn. and characterization of dansylaminotyrosyl-apo-**ACP** are reported. Dansylaminotyrosyl-apo-**ACP** was enzymically phosphopantetheinylated and acylated in vitro with an overall .apprx.30% yield of purified stearoyl-dansylaminotyrosyl-**ACP** starting from unmodified apo-**ACP**. The steady-state kinetic parameters kcat = 22 min-1 and KM = 2.7 .mu.M were detd. for reaction of stearoyl-dansylaminotyrosyl-**ACP** with stearoyl-**ACP** .DELTA.9-desaturase. These results show that dansylaminotyrosyl-**ACP** will function well for studying binding interactions with the .DELTA.9-desaturase and suggest similar possibilities for other **ACP**-dependent enzymes. The efficient in vivo phosphopantetheinylation of E. coli apo-**ACP** by coexpression with holo-**ACP** synthase in E. coli BL21(DE3) using fructose as the carbon source is also reported. (c) 2000 Academic Press.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:562278 CAPLUS
DOCUMENT NUMBER: 131:319468
TITLE: Role of Hydrophobic Partitioning in Substrate Selectivity and Turnover of the Ricinus communis Stearoyl Acyl Carrier Protein .DELTA.9 Desaturase
AUTHOR(S): **Haas, Jeffrey A.**; Fox, Brian G.
CORPORATE SOURCE: Institute for Enzyme Research Graduate School, University of Wisconsin, Madison, WI, 53705, USA
SOURCE: Biochemistry (1999), 38(39), 12833-12840
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Stearoyl acyl carrier protein .DELTA.9 desaturase (.DELTA.9D) uses a diiron center to catalyze the NADPH- and O2-dependent desatn. of stearoyl acyl carrier protein (**ACP**) to form oleoyl-**ACP**. The reaction of recombinant Ricinus communis .DELTA.9D with natural and nonnatural chain length acyl-ACPs was used to examine the coupling of the reconstituted enzyme complex, the specificity for position of double-bond insertion, the kinetic parameters for the desatn. reaction, and the selectivity for acyl chain length. The coupling of NADPH and O2 consumption and olefin prodn. was found to be maximal for 18:0-**ACP** , and the loss of coupling obsd. for the more slowly desatd. acyl-ACPs was attributed to autoxidn. of the electron-transfer chain. Anal. of steady-state kinetic parameters for desatn. of acyl-ACPs having various acyl chain lengths revealed that the Km values were similar (.apprx.2.5-fold difference) for 15:0-18:0-**ACP**, while the kcat

values increased by .apprx.26-fold for the same range of acyl chain lengths. A linear increase in log (kcat/Km) was obsd. upon lengthening of the acyl chain from 15:0- to 18:0-**ACP**, while no further increase was obsd. for 19:0-**ACP**. The similarity of the kcat/Km values for 18:0- and 19:0-ACPs and the retained preference for double-bond insertion at the .DELTA.9 position with 19:0-**ACP** (>98% desatn. at the .DELTA.9 position) suggest that the active-site channel past the diiron center can accommodate at least one more methylene group than is found in the natural substrate. The .DELTA..DELTA.Gbinding estd. from the change in kcat/Km for increasing substrate acyl-chain length was -3 kJ/mol per methylene group, similar to the value of -3.5 kJ/mol estd. for the hydrophobic partition of long-chain fatty acids (C-7 to C-21) from water to heptane [Smith, R., and Tanford, C. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 289-293]. Since the Km values are overall similar for all acyl-ACPs tested, the progressive increase in hydrophobic binding energy available from increased chain length is apparently utilized to enhance catalytic steps, which thus provides the underlying phys. mechanism for acyl chain selectivity obsd. with .DELTA.9D.

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:290961 CAPLUS

DOCUMENT NUMBER: 131:141351

TITLE: Catalytic and spectroscopic studies of acyl-**ACP** desaturation: the unique contributions of protein and lipid to enzymatic catalysis

AUTHOR(S): Broadwater, John A.; **Haas, Jeffrey A.**; Achim, Catalina; Munck, Eckard; Ai, Jingyuan; Sanders-Loehr, Joann; Loehr, Thomas M.; Fox, Brian G.

CORPORATE SOURCE: The Institute for Enzyme Research and Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, 53705, USA

SOURCE: Biomedical and Health Research (1999), 27(Enzymatic Mechanisms), 162-175
CODEN: BIHREN; ISSN: 0929-6743

PUBLISHER: IOS Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The diiron enzyme stearoyl-acyl carrier protein (**ACP**) .DELTA.9 desaturase catalyzes the NAD(P)H- and O2-dependent desatn. of 18:0-**ACP** to give 18:1-**ACP**. The reconstituted catalytic system is a four protein complex consisting of ferredoxin reductase, [2Fe-2S] ferredoxin, the terminal homodimeric desaturase, and the fourth protein component, which is the substrate, 18:0-**ACP**. Genetic engineering, coexpression and fermn. have been required to produce sufficient quantities of recombinant acyl-ACPs for expanded studies of enzymic desatn. Among the well-characterized diiron enzymes, the authors have found that diferrous .DELTA.9D is distinguished by an extremely slow rate of single turnover autoxidn., yet reacts with 18:0-**ACP** under steady state conditions to give 18:1-**ACP** with >103 increase in oxidn. rate. Moreover, previous studies indicated that the desaturase exhibits .apprx.102 fold higher Vmax for 18:0-**ACP** as compared to 16:0-**ACP**, while the apparent Km-values were indistinguishable. These studies suggest the importance of the acyl portion of the substrate in activating the enzyme for catalysis. This suggestion is substantiated by our recent studies of complexes of diferrous enzyme, 18:0-**ACP**, and O2. Optical and resonance Raman studies revealed an intermediate with absorption at .apprx.700 nm, an O-O sym. vibration at 898 cm-1, and relatively long lifetime (t1/2 .apprxeq. 23 min). These spectral characteristics suggest a quasi-stable peroxy differic intermediate. Mossbauer studies revealed an antiferromagnetically coupled, sym. intermediate with an isomer shift of .apprx.0.85 mm/s; this intermediate value of isomer shift has not been obsd. in any previous studies of diiron enzymes. Under single turnover

conditions, the complex decays to the resting state without prodn. of 18:1-**ACP**. However, since no detectable levels of H2O2 were obtained from this reaction, an oxidase reaction arising from reaction at one of the two available diiron centers is proposed. By coupling formation of a relatively stable peroxy diferric intermediate with subsequent electron and proton transfers, this reaction provides a catalytic precedent for the "alternative oxidase" activity recently assigned to a membrane diiron enzyme in thermogenic plants and trypanosomes.

REFERENCE COUNT: 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:467569 BIOSIS
DOCUMENT NUMBER: PREV199900467569
TITLE: Steady-state kinetic studies of stearoyl-**ACP** DELTA9 desaturase: Role of hydrophobic partitioning in substrate selectivity and turnover.
AUTHOR(S): **Haas, Jeffrey A.** [Reprint author]; Fox, Brian G. [Reprint author]
CORPORATE SOURCE: Department of Biochemistry, Institute for Enzyme Research, University of Wisconsin, Madison, WI, 53705, USA
SOURCE: Journal of Inorganic Biochemistry, (April 30, 1999) Vol. 74, No. 1-4, pp. 151. print.
Meeting Info.: 9th International Conference on Biological Inorganic Chemistry. Minneapolis, Minnesota, USA. July 11-16, 1999.
CODEN: JIBIDJ. ISSN: 0162-0134.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Nov 1999
Last Updated on STN: 9 Nov 1999

=> d his

(FILE 'HOME' ENTERED AT 11:35:15 ON 15 JAN 2004)

FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT 11:35:53 ON 15 JAN 2004

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L1          0 S ACYL CARRIER PROTEIN AND NON-RADIOACTIVE LABEL?
L2          0 S ACYL CARRIER PROTEIN AND CHROMO? LABEL?
L3         17 S ACYL CARRIER PROTEIN AND CHROMOPHORE
L4          5 DUP REM L3 (12 DUPLICATES REMOVED)
L5         35 S ACYL CARRIER PROTEIN AND CHEMICAL MODIFICATION
L6         19 DUP REM L5 (16 DUPLICATES REMOVED)
L7         19 FOCUS L6 1-
L8          0 S ACYL CARRIER PROTEIN AND APO-ACYL CARRIER PROTEIN AND HOLO-AC
L9         79 S (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACY
L10        41 DUP REM L9 (38 DUPLICATES REMOVED)
L11        41 FOCUS L10 1-
L12        37 S (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACY
L13        23 DUP REM L12 (14 DUPLICATES REMOVED)
L14        23 FOCUS L13 1-
L15        20 S (ACYL CARRIER PROTEIN OR APO-ACYL CARRIER PROTEIN OR HOLO-ACY
L16        10 DUP REM L15 (10 DUPLICATES REMOVED)
L17        10 FOCUS L16 1-
L18        13 S ACYL CARRIER PROTEIN AND (DANSYL OR FLUORESCEIN OR RHODAMINE
L19         6 DUP REM L18 (7 DUPLICATES REMOVED)
L20         0 S FOX G BRIAN/AU
              E L3
L21       144 S FOX BRIAN G/AU
L22        89 DUP REM L21 (55 DUPLICATES REMOVED)
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L23           20 S L22 AND ACYL CARRIER PROTEIN
L24            1 S L23 AND LABEL
L25            0 S HAAS A JEFFREY/AU
L26           14 S HAAS JEFFREY A/AU
L27            7 DUP REM L26 (7 DUPLICATES REMOVED)
L28            6 S L27 AND ACP

=> log
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                        ENTRY    SESSION
FULL ESTIMATED COST                                    363.29     363.50

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                        ENTRY    SESSION
CA SUBSCRIBER PRICE                                     -9.01      -9.01

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=> file medline caplus biosis embase scisearch
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 10:47:52 ON 16 JAN 2004

FILE 'CAPLUS' ENTERED AT 10:47:52 ON 16 JAN 2004
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FILE 'SCISEARCH' ENTERED AT 10:47:52 ON 16 JAN 2004
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=> s (acyl carrier protein or ACP) and NMR
L1          331 (ACYL CARRIER PROTEIN OR ACP) AND NMR

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          154 DUP REM L1 (177 DUPLICATES REMOVED)

=> s l2 and 15N
L3            8 L2 AND 15N

=> d l3 1-8 ibib ab

L3   ANSWER 1 OF 8     MEDLINE on STN
ACCESSION NUMBER:   2003474811     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 14536035
TITLE:              Backbone 1H, 15N, and 13C resonance assignments
                    of the Helicobacter pylori acyl carrier
                    protein.
AUTHOR:             Park Sung Jean; Kim Ji-Sun; Son Woo-Sung; Ahn Hee Chul; Lee
                    Bong Jin
CORPORATE SOURCE:   Research Institute of Pharmaceutical Sciences, College of
                    Pharmacy, Seoul National University, Seoul 151-742, Korea..
                    lbj@nmr.snu.ac.kr
SOURCE:             Journal of biochemistry and molecular biology, (2003 Sep
                    30) 36 (5) 505-7.
                    Journal code: 9702084. ISSN: 1225-8687.
PUB. COUNTRY:       Korea (South)
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200311
ENTRY DATE:         Entered STN: 20031011
                    Last Updated on STN: 20031219
                    Entered Medline: 20031117
AB   One of the small proteins from Helicobacter pylori, acyl
     carrier protein (ACP), was investigated by
     NMR. ACP is related to various cellular processes,
     especially with the biosynthesis of fatty acid. The basic NMR
     resonance assignment is a prerequisite for the validation of a
     heterologous protein interaction with ACP in H. pylori. Here,
     the results of the backbone (1)H, (15)N, and (13)C resonance assignments
     of the H. pylori ACP are reported using double- and
     triple-resonance techniques. About 97% of all of the (1)HN, (15)N,
     (13)CO, (13)Calpha, and (13)Cbeta resonances that cover 76 of the 78
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non-proline residues are clarified through sequential- and specific-assignments. In addition, four helical regions were clearly identified on the basis of the resonance assignments.

L3 ANSWER 2 OF 8 MEDLINE on STN
ACCESSION NUMBER: 95392401 MEDLINE
DOCUMENT NUMBER: 95392401 PubMed ID: 7663354
TITLE: Amide exchange rates in Escherichia coli **acyl carrier protein**: correlation with protein structure and dynamics.
AUTHOR: Andrec M; Hill R B; Prestegard J H
CORPORATE SOURCE: Department of Chemistry, Yale University, New Haven, Connecticut 06511, USA.
CONTRACT NUMBER: GM33225 (NIGMS)
SOURCE: PROTEIN SCIENCE, (1995 May) 4 (5) 983-93.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951020
Last Updated on STN: 19970203
Entered Medline: 19951010

AB The **acyl carrier protein** (**ACP**) of Escherichia coli is a 77-amino acid, highly negatively charged three-helix protein that plays a central role in fatty acid biosynthesis. Previous **NMR** studies have suggested the presence of multiple conformations and marginally stable secondary structural elements. The stability of these elements is now examined by monitoring amide exchange in apo-**ACP** using **NMR**-based methods. Because **ACP** exhibits many rapid exchange rates, application of traditional isotope exchange methods is difficult. In one approach, heteronuclear correlation experiments with pulsed field-gradient coherence selection have reduced the time needed to collect two-dimensional 1H-**15N** correlation spectra to the point where measurement of exchange of amide protons for deuterium on the timescale of minutes can be made. In another approach, water proton selective inversion-exchange experiments were performed to estimate the exchange rates of protons exchanging on timescales of less than a second. Backbone amide protons in the region of helix II were found to exchange significantly more rapidly than those in helices I and III, consistent with earlier structural models suggesting a dynamic disruption of the second helix. Highly protected amides occur on faces of the helices that may pack into a hydrophobic core present in a partially disrupted state.

L3 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:949685 CAPLUS
DOCUMENT NUMBER: 139:193518
TITLE: Letter to the Editor: Backbone 1H, **15N** and 13C resonance assignments of the Staphylococcus aureus **acyl carrier protein** synthase (AcpS)
AUTHOR(S): Liu, Dingjiang; Black, Todd; Macinga, David R.; Palermo, Robert; Wyss, Daniel F.
CORPORATE SOURCE: Department of Structural Chemistry, Schering-Plough Research Institute, Kenilworth, NJ, 07033, USA
SOURCE: Journal of Biomolecular NMR (2002), 24(3), 273-274
CODEN: JBNME9; ISSN: 0925-2738
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The sequence-specific backbone resonance assignments of Staphylococcus aureus AcpS is described. S. aureus AcpS has 49% and 37% sequence identity to Bacillus subtilis AcpS and Streptococcus pneumoniae AcpS,

resp. The native S. aureus AcpS contains 119 amino acids with a mol. wt. of 13.6 kDa per monomer.
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:18255 CAPLUS
DOCUMENT NUMBER: 134:125015
TITLE: Single-transition coherence transfer by adiabatic cross polarization in **NMR**
AUTHOR(S): Eykyn, Thomas R.; Ferrage, Fabien; Winterfors, Emanuel; Bodenhausen, Geoffrey
CORPORATE SOURCE: Departement de Chimie, associe au CNRS, Ecole Normale Superieure, Paris, 75231, Fr.
SOURCE: ChemPhysChem (2000), 1(4), 217-221
Published in: Angew. Chem., Int. Ed., 39(24)
CODEN: CPCHFT; ISSN: 1439-4235
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A new method for the spin-state-selective coherence transfer is introduced based on the principles of adiabatic cross polarization. The method is called single-transition adiabatic cross polarization (ST-**ACP**). It was analyzed theor. for a scalar-coupled 2-spin 1/2 system. An exptl. test was also developed which reveals the violations from adiabacity. **NMR** measurements were performed on **15N**-enriched samples of tert-butoxycarbonyl-protected glycine. The reversibility of the coherence transfer serves as the exptl. criterion for the adiabacity.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:323556 CAPLUS
DOCUMENT NUMBER: 122:100843
TITLE: Simultaneous collection of two transverse **15N** relaxation pathways in isotopically labeled proteins
AUTHOR(S): Tolman, Joel R.; Prestegard, James H.
CORPORATE SOURCE: Dep. Chem., Yale Univ., New Haven, CT, 06511, USA
SOURCE: Journal of Magnetic Resonance, Series B (1995), 106(1), 97-100
CODEN: JMRBE5; ISSN: 1064-1866
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Simultaneous fit of a complete time course allows detn. of 3 relaxation rates, .rho.S, .rho.IS, and .delta.SIS, for each resolved amide resonance. The measurement of these rates is a necessary step for ultimately sepg. the magnitudes of the dipolar, chem. shift anisotropy (CSA), and dipolar-CSA contributions to the relaxation of the amide nitrogens in a protein backbone.

L3 ANSWER 6 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:528372 BIOSIS
DOCUMENT NUMBER: PREV200000528372
TITLE: Direct measurement of 1H-1H dipolar couplings in proteins: A complement to traditional NOE measurements.
AUTHOR(S): Tian, F.; Fowler, C. A.; Zartler, E. R.; Jenney, F. A., Jr.; Adams, M. W.; Prestegard, J. H. [Reprint author]
CORPORATE SOURCE: Complex Carbohydrate Research Center, University of Georgia, Athens, GA, 30602-4712, USA
SOURCE: Journal of Biomolecular NMR, (September, 2000) Vol. 18, No. 1, pp. 23-31. print.
CODEN: JBNME9. ISSN: 0925-2738.
DOCUMENT TYPE: Article
LANGUAGE: English

ENTRY DATE: Entered STN: 6 Dec 2000
Last Updated on STN: 11 Jan 2002

AB An intensity-based constant-time COSY (CT-COSY) method is described for measuring 1H-1H residual dipolar couplings of proteins in weakly aligned media. For small proteins, the overall sensitivity of this experiment is comparable to the NOESY experiment. In cases where the 1H-1H distances are defined by secondary structure, such as 1Halpha-1HN and 1HN-1HN sequential distances in alpha-helices and beta-sheets, these measurements provide useful orientational constraints for protein structure determination. This experiment can also be used to provide distance information similar to that obtained from NOE connectivities once the angular dependence is removed. Because the measurements are direct and non-coherent processes, such as spin diffusion, do not enter, the measurements can be more reliable. The 1/r3 distance dependence of directly observed dipolar couplings, as compared with the 1/r6 distance dependence of NOEs, also can provide longer range distance information at favorable angles. A simple 3D, **15N** resolved version of the pulse sequence extends the method to provide the improved resolution required for application to larger biomolecules.

L3 ANSWER 7 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1995:455516 BIOSIS
DOCUMENT NUMBER: PREV199598469816
TITLE: Overexpression purification, and characterization of Escherichia coli **acyl carrier protein** and two mutant proteins.
AUTHOR(S): Hill, R. Blake; MacKenzie, Kevin R.; Flanagan, John M.; Cronan., John E., Jr.; Prestegard, James H. [Reprint author]
CORPORATE SOURCE: Dep. Chem., Yale Univ., New Haven, CT 06520, USA
SOURCE: Protein Expression and Purification, (1995) Vol. 6, No. 4, pp. 394-400.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Oct 1995
Last Updated on STN: 27 Oct 1995

AB A synthetic gene of 237 bases encoding the 77-residue **acyl carrier protein** (**ACP**) from Escherichia coli, along with two mutant genes, **ACP**-I54V and **ACP**-A59V, were subcloned into the pET11a-pLysS E. coli overexpression system under the control of the bacteriophage T7 promoter. This efficient expression system and a simplified purification protocol yielded more than 120 mg/l of pure protein. The construct produced a mixture of holo-**ACP** and apo-**ACP** and two HPLC procedures were developed to separate the two species. This overexpression system allows cost-effective growths of 13C- and **15N**-labeled protein for structural and other studies on **ACP**. In the course of the work on the mutants of **ACP** , an apparent homologous recombination event led, in one case, to reversion to a wild-type protein, suggesting that precautions to prevent such reversion should be taken.

L3 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1993:406349 BIOSIS
DOCUMENT NUMBER: PREV199396072074
TITLE: **NMR** study of kinetic HH/DD/DH/DD isotope effects on the tautomerism of acetylporphyrin: Evidence for a stepwise double proton transfer.
AUTHOR(S): Schlabach, Martin; Limbach, Hans-Heinrich [Reprint author]; Bunnenberg, Edward; Shu, Arthur Y. L.; Tolf, Bo-Ragnar; Djerassi, Carl
CORPORATE SOURCE: Institut Organische Chemie der Freien Universitaet Berlin, Takustr. 3, W-1000 Berlin 33, Germany
SOURCE: Journal of the American Chemical Society, (1993) Vol. 115, No. 11, pp. 4554-4565.

CODEN: JACSAT. ISSN: 0002-7863.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Sep 1993
Last Updated on STN: 8 Sep 1993

AB The kinetic HH/HD/DH/DD isotope effects of an intramolecular reversible nondegenerate double proton transfer reaction are described. The molecule studied is 8-acetyl-3,13,17-tris(2-(methoxycarbonyl)ethyl)-2,7,12,18-tetramethyl-(21H,23H)-porphyrin (acetylporphyrin, **ACP**) dissolved in CD-2Cl-2. As a thermodynamic and kinetic method we used dynamic 1H and 13C **NMR** spectroscopy of suitably 2H, **15N**, and 13C labeled **ACP**. The **15N** label was introduced at the 21, 23, and 24 positions, the 13C label at the 6- and 7-methyl positions, and 2H at the central proton sites, respectively. The syntheses of these compounds are reported. **ACP** exists in two tautomeric forms of different energy which interconvert rapidly at room temperature and slowly at 200 K with respect to the **NMR** time scale. The tautomer with a proton located on the acetylpyrrole ring has the higher energy. The equilibrium constant for interconversion of the tautomers is given by K = 1.14 times exp(-5.82 kJ mol-1/RT). Equilibrium isotope effects on the tautomerism of **ACP** are not observed within the margin of error. Whereas the tautomerism of **ACP**-HH and of **ACP**-DD can each be described by one rate constant, we find two different rate constants for **ACP**-HD: one for the HD and one for the DH reaction, respectively. In the HD reaction, an H atom jumps to the acetyl-substituted pyrrole ring; in the DH reaction, the D atom migrates. We obtain the following kinetic results: k-HH = 10-10.4 exp(-40 kJ mol-1/RT), 230 K ltoreq T ltoreq 327 K, k-HH(298) simeq 2840 s-1, k-HD = 10-11.3 exp(-52 kJ mol-1/RT), 266 K ltoreq T ltoreq 311 K, k-HD(298) simeq 180 s-1, k-DH = 10-10.0 exp(-41 kJ mol-1/RT), 252 K ltoreq T ltoreq 312 K, k-DH(298) simeq 670 s-1, k-DD = 10-11.5 exp(-53.5 kJ mol-1/RT), 273 K ltoreq T ltoreq 380 K, k-DD(298) simeq 150 s-1, with the kinetic isotope effects of k-HH/k-HD simeq 16, k-HH/k-DH simeq 4, k-HH/k-DD simeq 19, k-HD/k-DD simeq 1.2, k-DH simeq 4.5 at 298 K. These results are modeled in terms of a stepwise proton transfer. The HD and the DD reaction are characterized by similar rate constants because in both cases, a deuterium is transferred in the rate-determining step. The implications of these results to other chemically and biologically relevant multiple proton-transfer systems are discussed.

=> s l3 and Escherichia coli
L4 3 L3 AND ESCHERICHIA COLI

=> d l4 1-3 ibib ab

L4 ANSWER 1 OF 3 MEDLINE on STN
ACCESSION NUMBER: 95392401 MEDLINE
DOCUMENT NUMBER: 95392401 PubMed ID: 7663354
TITLE: Amide exchange rates in **Escherichia coli acyl carrier protein**: correlation with protein structure and dynamics.
AUTHOR: Andrec M; Hill R B; Prestegard J H
CORPORATE SOURCE: Department of Chemistry, Yale University, New Haven, Connecticut 06511, USA.
CONTRACT NUMBER: GM33225 (NIGMS)
SOURCE: PROTEIN SCIENCE, (1995 May) 4 (5) 983-93.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951020
Last Updated on STN: 19970203

Entered Medline: 19951010

AB The **acyl carrier protein** (**ACP**) of **Escherichia coli** is a 77-amino acid, highly negatively charged three-helix protein that plays a central role in fatty acid biosynthesis. Previous **NMR** studies have suggested the presence of multiple conformations and marginally stable secondary structural elements. The stability of these elements is now examined by monitoring amide exchange in apo-**ACP** using **NMR**-based methods. Because **ACP** exhibits many rapid exchange rates, application of traditional isotope exchange methods is difficult. In one approach, heteronuclear correlation experiments with pulsed field-gradient coherence selection have reduced the time needed to collect two-dimensional 1H-**15N** correlation spectra to the point where measurement of exchange of amide protons for deuterium on the timescale of minutes can be made. In another approach, water proton selective inversion-exchange experiments were performed to estimate the exchange rates of protons exchanging on timescales of less than a second. Backbone amide protons in the region of helix II were found to exchange significantly more rapidly than those in helices I and III, consistent with earlier structural models suggesting a dynamic disruption of the second helix. Highly protected amides occur on faces of the helices that may pack into a hydrophobic core present in a partially disrupted state.

L4 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:528372 BIOSIS
DOCUMENT NUMBER: PREV200000528372
TITLE: Direct measurement of 1H-1H dipolar couplings in proteins: A complement to traditional NOE measurements.
AUTHOR(S): Tian, F.; Fowler, C. A.; Zartler, E. R.; Jenney, F. A., Jr.; Adams, M. W.; Prestegard, J. H. [Reprint author]
CORPORATE SOURCE: Complex Carbohydrate Research Center, University of Georgia, Athens, GA, 30602-4712, USA
SOURCE: Journal of Biomolecular NMR, (September, 2000) Vol. 18, No. 1, pp. 23-31. print.
CODEN: JBNME9. ISSN: 0925-2738.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Dec 2000
Last Updated on STN: 11 Jan 2002

AB An intensity-based constant-time COSY (CT-COSY) method is described for measuring 1H-1H residual dipolar couplings of proteins in weakly aligned media. For small proteins, the overall sensitivity of this experiment is comparable to the NOESY experiment. In cases where the 1H-1H distances are defined by secondary structure, such as 1Halpha-1HN and 1HN-1HN sequential distances in alpha-helices and beta-sheets, these measurements provide useful orientational constraints for protein structure determination. This experiment can also be used to provide distance information similar to that obtained from NOE connectivities once the angular dependence is removed. Because the measurements are direct and non-coherent processes, such as spin diffusion, do not enter, the measurements can be more reliable. The 1/r3 distance dependence of directly observed dipolar couplings, as compared with the 1/r6 distance dependence of NOEs, also can provide longer range distance information at favorable angles. A simple 3D, **15N** resolved version of the pulse sequence extends the method to provide the improved resolution required for application to larger biomolecules.

L4 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1995:455516 BIOSIS
DOCUMENT NUMBER: PREV199598469816
TITLE: Overexpression purification, and characterization of **Escherichia coli acyl carrier protein** and two mutant proteins.
AUTHOR(S): Hill, R. Blake; MacKenzie, Kevin R.; Flanagan, John M.; Cronan., John E., Jr.; Prestegard, James H. [Reprint

author]
CORPORATE SOURCE: Dep. Chem., Yale Univ., New Haven, CT 06520, USA
SOURCE: Protein Expression and Purification, (1995) Vol. 6, No. 4, pp. 394-400.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
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AB A synthetic gene of 237 bases encoding the 77-residue **acyl carrier protein** (**ACP**) from **Escherichia coli**, along with two mutant genes, **ACP**-I54V and **ACP**-A59V, were subcloned into the pET11a-pLysS E. coli overexpression system under the control of the bacteriophage T7 promoter. This efficient expression system and a simplified purification protocol yielded more than 120 mg/l of pure protein. The construct produced a mixture of holo-**ACP** and apo-**ACP** and two HPLC procedures were developed to separate the two species. This overexpression system allows cost-effective growths of 13C- and **15N**-labeled protein for structural and other studies on **ACP**. In the course of the work on the mutants of **ACP**, an apparent homologous recombination event led, in one case, to reversion to a wild-type protein, suggesting that precautions to prevent such reversion should be taken.

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FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 10:47:52 ON 16 JAN 2004
L1 331 S (ACYL CARRIER PROTEIN OR ACP) AND NMR
L2 154 DUP REM L1 (177 DUPLICATES REMOVED)
L3 8 S L2 AND 15N
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☐	L13	apo-acyl carrier protein and non-radiactive label	0
☐	L12	apo-acyl carrier protein and radiactive label.clm.	0
☐	L11	acyl carrier protein and radiactive label.clm.	0
☐	L10	acyl carrier protein and non-radiactive label.clm.	0
☐	L9	ACP and radiactive label.clm.	0
☐	L8	ACP and non-radiactive label.clm.	0
☐	L7	acyl carrier protein and non-radioactive label	2
☐	L6	l3 and chemical modification	16
☐	L5	acyl carrier protein and modication.clm.	0
☐	L4	labeled acyl carrier protein.clm.	0
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☐	L1	acyl carrier protein and modification	835

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